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Sexual selection has minimal impact on effective population sizes in species with high rates of random offspring mortality: an empirical demonstration using fitness distributions

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Abstract

The effective population size (N_e) is a fundamental parameter in population genetics that influences the rate of loss of genetic diversity. Sexual selection has the potential to reduce N_e by causing the sex-specific distributions of individuals that successfully reproduce to diverge. To empirically estimate the effect of sexual selection on N_e , we obtained fitness distributions for males and females from an outbred, laboratory-adapted population of *Drosophila melanogaster*. We observed strong sexual selection in this population (the variance in male reproductive success was ~14 times higher than that for females), but found that sexual selection had only a modest effect on N_e , which was 75% of the census size. This occurs because the substantial random offspring mortality in this population diminishes the effects of sexual selection on N_e , a result that necessarily applies to other high fecundity species. The inclusion of this random offspring mortality creates a scaling effect that reduces the variance/mean ratios for male and female reproductive success and causes them to converge. Our results demonstrate that measuring reproductive success without considering offspring mortality can underestimate N_e and overestimate the genetic consequences of sexual selection. Similarly, comparing genetic diversity among different genomic components may fail to detect strong sexual selection.

Keywords

Selection; reproductive success; juvenile mortality; genetic variation; autosomes; sex chromosomes

Introduction

The effective size of a population (N_e) is a fundamental parameter in population genetics that determines the rate at which genetic drift purges genetic diversity from a population. N_e is defined as the size of a mathematically tractable “ideal population” (with random mating, no selection, equal sex ratio, Poisson distribution of family sizes and constant population size of adults) that would lose genetic variation at the same rate as a more complex, real population. Nearly all deviations of a natural population from an ideal population make N_e smaller than the census size (N), and the proportionate difference in N_e compared to N (N_e/N) is a useful measure of how these deviations change the rate of decay in genetic diversity.

The N_e of a population can be determined using the variance in reproductive success divided by its mean for each sex (Crow and Morton 1955). In most systems, sexual selection influences male reproductive success to a much larger degree than female reproductive success (e.g. Bateman 1948; reviewed in Andersson 1994). This increases the variance in male reproductive success compared to a Poisson distribution, especially by increasing the number of males with zero reproductive success (Crow and Morton 1955). Because sexual selection causes the distributions of reproductive success (i.e. the variance/mean ratios) for the two sexes to diverge, it can consequently lower the overall N_e of a population and increase the rate at which genetic diversity is lost. Accurate distributions of reproductive success for both males and females can be used to assess the impact of sexual selection on the effective size of a population.

The estimates of reproductive success needed to determine N_e are challenging to obtain because they require not only the number of offspring produced by each individual, but also the mortality of those offspring before they reach reproductive age. This is especially difficult for natural populations, as the fecundity, paternity, and survival of a large sample of individuals must be tracked, and offspring may disperse to unsurveyed locations, preventing accurate measures of mortality. For example, in long-term studies of iteroparous bird species such as great tits (McCleery et al. 2004), flycatchers (Merilä and Sheldon 2000), and buzzards (Krüger and Lindström 2001), detailed measures of lifetime reproductive success and egg-to-fledgling survival have been accurately obtained for both sexes. The dispersal of juveniles outside the study locations, however, makes it impractical to include later measures of offspring mortality. Similarly, Kruuk et al. (2000) estimated lifetime fitness of male and female red deer from a population on the Isle of Rum. This population is not fully enclosed, however, making it difficult to follow the fate of all offspring. As we demonstrate below, offspring mortality is a critical component of N_e calculations because it reduces the variance in reproductive success for both sexes. This effect is more extreme for the sex experiencing stronger sexual selection (usually males), causing the variance/mean ratios for

the two sexes to converge. Fitness measures obtained using offspring counts before much mortality has occurred can be misleading.

In laboratory-adapted populations, it is possible to measure all the necessary components of fitness in each sex and use them to calculate N_e . We estimated the sex-specific distributions of reproductive success for a large and outbred laboratory population of *Drosophila melanogaster* (LH_M) that has adapted to the same, highly competitive environment for over 400 generations. We also estimated distributions of juvenile survival to verify that there are no differences in male and female survival that could affect our measures of reproductive success. We then used our distributions of reproductive success to demonstrate how sexual selection and random offspring mortality impact the N_e of the two sexes, the population as a whole, and the different components of the genome.

Calculating N_e for males and females using distributions of reproductive success

Here we consider the case of separate sexes and non-overlapping generations, as occurs in most laboratory populations of *Drosophila*, wild populations of many annual plants, *Antechinus* marsupials, and periodical cicadas (Alexander and Moore 1962; Lazenby-Cohen and Cockburn 1988; Kraaijeveld-Smit et al. 2002). We focus on the “inbreeding effective size” (the size of an ideal population that would have the same level of inbreeding as the natural, non-ideal population). Other criteria for determining N_e are also possible (e.g., based on variance in allele frequencies across generations, or coalescence times), but it should be noted that all methods produce similar results at equilibrium when N is large and constant (Hill 1972; Whitlock and Barton 1997).

In an ideal population, variation in fitness among individuals is assumed to follow a Poisson distribution, which has a variance/mean ratio of 1. In natural populations, however, the variance in fitness often exceeds the mean, causing N_e to be reduced compared to N . Within each sex, the value of N_e/N is determined by the variance/mean ratio for reproductive success, σ^2_W/μ_W :

$$\frac{N_{e(\text{sex})}}{N_{\text{sex}}} = \frac{2}{\left(1 + \left[\sigma^2_{W(\text{sex})}/\mu_{W(\text{sex})}\right]\right)}, \quad (1)$$

where the subscript “sex” is “M” for males or “F” for females, the population is randomly mating, and the population size is assumed to be neither increasing nor decreasing (Crow and Morton 1955). Note that when $\sigma^2_W/\mu_W=1$ then $N_{e(\text{sex})}=N_{\text{sex}}$, but when $\sigma^2_W/\mu_W>1$ then $N_{e(\text{sex})}<N_{\text{sex}}$.

Incorporating offspring mortality into sex-specific measures of N_e

When the population size is stable, as is assumed in the above equation, the average number of offspring per individual must be two each generation. When the average reproductive success (measured before much mortality has occurred) is greater than two offspring per individual, Crow and Morton (1955) calculated an adjusted σ^2_W/μ_W that applies random

(binomial) offspring mortality to bring the mean down to two offspring and the population back to N surviving adults:

$$\sigma^2_{W(\text{sex})}/\mu_{W(\text{sex})} | (\mu_{W(\text{sex})}' = 2) = 2 \left(\left\{ \left[\sigma^2_{W(\text{sex})}' / \mu_{W(\text{sex})}' \right] - 1 \right\} / \mu_{W(\text{sex})}' \right) + 1, \quad (2)$$

where a prime denotes the mean and variance in number of younger offspring before random mortality culls the number of surviving offspring back down to N . Inspection of equation (2) reveals that this correction has a larger effect on the sex with a higher $\sigma^2_{W(\text{sex})}'/\mu_{W(\text{sex})}'$.

Substituting equation (2) into (1) yields:

$$\frac{N_{e(\text{sex})}}{N_{\text{sex}}} = \frac{\mu_{W(\text{sex})}'}{\left(\mu_{W(\text{sex})}' - 1 + \left[\sigma^2_{W(\text{sex})}' / \mu_{W(\text{sex})}' \right] \right)}. \quad (3)$$

This result is identical to that of Lande and Barrowclough (1987), except that their numerator is decremented by one ($\mu_{W(\text{sex})}' - 1$) compared to that of Crow and Morton ($\mu_{W(\text{sex})}'$). The adjustment in equation (2) can be used to account for random offspring mortality in any numerically stable population with non-overlapping generations (Crow and Morton 1955), and can therefore be applied to early offspring counts to more accurately calculate N_e .

Calculating N_e of a population and its different genomic components

In both natural and laboratory populations, the sex-specific variance in reproductive success ($\sigma^2_{W(\text{sex})}$) can exceed its mean, particularly when sexual selection is operating. When equation (3) is applied to each sex, N_e will be smaller for the sex experiencing stronger sexual selection (usually males) due to a larger variance/mean ratio, σ^2_W/μ_W . When one sex has a smaller N_e compared to the other, this disparity reduces the overall N_e of the population (Wright 1931). Once $N_{e(M)}$ and $N_{e(F)}$ are known, the effective size of the population as a whole, relative to N , can be calculated as:

$$\frac{N_e}{N} = \frac{\left(4N_{e(M)} N_{e(F)} \right)}{N \left(N_{e(M)} + N_{e(F)} \right)}, \quad (4)$$

where symbols lacking the subscripts “M” or “F” refer to the whole population (Crow and Kimura 1970). Inspection of equation (4) demonstrates that the value of N_e/N is maximized at 1 when $N_{e(M)} = N_{e(F)} = N/2$.

The value of N_e/N can differ substantially for different genomic components. For the autosomes (A), which fit the assumptions used to calculate N_e/N above, $N_{e(A)}/N = N_e/N$. For the X, which is 3/4ths as numerous as the autosomes and spends 2/3rds of its time in females, N_e/N is given by:

$$\frac{N_{e(X)}}{N} = \frac{\left(9N_{e(F)}N_{e(M)}\right) / \left(2N_{e(F)} + N_{e(M)}\right)}{N}, \quad (5)$$

(Wright 1931). For the Y, which is hemizygous and resides exclusively in males:

$$\frac{N_{e(Y)}}{N} = \frac{N_{e(M)}}{N} / 2, \quad (6)$$

(Hedrick 2000). Finally, for a cytoplasmically propagated mitochondria or endosymbiont (C):

$$\frac{N_{e(C)}}{N} = \frac{N_{e(F)}}{N} / 2, \quad (7)$$

(Hedrick 2000).

Materials and Methods

(a) Maintenance of the LH_M laboratory-adapted *D. melanogaster* population

The population is maintained via transfer between three sequential sets (representing life stages) of 56 vials each generation: juvenile competition vials, adult competition vials, and oviposition vials. A generation begins when adults from the previous generation (16 males and 16 females per vial) lay eggs in 56 oviposition vials. After 18 h the adults are removed and these vials become the juvenile competition vials of the next generation. The eggs in these vials (~40,000 eggs in total, or ~715 eggs/vial) are then culled by individually counting out ~180 *in situ* eggs per vial and discarding the remainder, leaving ~10,000 eggs in total distributed among the 56 vials. Here the eggs hatch, compete as larvae, pupate, eclose and mature into young adults. Most females (96-99%) have mated at least once by the time they exit the juvenile competition vials (Long et al. 2010). To start the next life history stage, adult flies from the juvenile competition vials are mixed between vials 11.25 days after egg deposition and are transferred into adult competition vials. Density in the adult competition vials is reduced to only 16 males and 16 females per vial. In the adult competition vials, females compete for a limited supply of live yeast (6 mg/vial) and males compete to mate females and fertilize their eggs. Most females (90-100%) remate at least once during the two-day adult competition phase of the lifecycle (Orteiza et al. 2005). After 2 days in the adult competition vials, the flies are transferred to unyeasted oviposition vials for 18 h, and only the eggs produced during this time (randomly culled to a density of ~180 eggs per vial) are used to begin the next generation. At the time of our experiments the LH_M population had been maintained under these conditions for over 400 generations. See Rice et al. (2005; 2006) for a more detailed description of the LH_M culture protocol.

(b) Measuring juvenile survival

To measure juvenile survival (i.e. juvenile fitness), we set up 138 egg-laying chambers, each containing 25 males and 25 females from the LH_M population and a petri dish filled with food medium on which the females could oviposit. After 18 h, we randomly collected 60 eggs from each dish, and measured egg-to-adult viability by placing these 60 eggs into a juvenile competition vial containing 120 competitor eggs from a replica of the LH_M population (LH_M-bw) into which a recessive brown-eyed marker (*bw*) had been introgressed through repeated backcrossing. In total, 138 juvenile competition vials were set up. The numbers of males and females that eclosed from the 60 target eggs per vial were scored 11.25 days after the eggs were laid and taken as a measure of sex-specific juvenile survival (Friberg et al. 2011). The time at which egg-to-adult viability was scored corresponds to the time when adults are transferred from the juvenile competition to the adult competition vials in the LH_M population (Rice et al. 2005; 2006).

(c) Measuring adult reproductive success

We measured reproductive success for a total of 100 males and 100 females over 10 experimental replicates, with 10 males and 10 females surveyed in each replicate. Each replicate began by setting up 35 juvenile competition vials that contained a single egg from the LH_M population combined with 174 competitor eggs from the LH_M-bw population; the individuals that we screened thus experienced standard levels of larval competition. Corresponding to the beginning of the adult competition phase of the lifecycle, these 35 vials were surveyed 11.25 days later, and 10 vials each were selected in which the LH_M egg developed into an adult male or female. We then measured reproductive success for the 10 males and 10 females over the complete lifecycle of the LH_M population.

We measured reproductive success for each female (100 females total) as follows. On Day 12.25, we set up a single adult competition vial containing the LH_M female, 15 competitor LH_M-bw females (raised with the focal LH_M female), and 16 LH_M-bw males (also raised with the focal LH_M female). On Day 14.25, we transferred the LH_M female to an individual oviposition vial for 18 h, and counted the number of offspring that eclosed from this vial 12 days later. Since only eggs laid during this 18 h oviposition phase of the lifecycle are used to propagate the LH_M population, offspring produced during this period represent a female's lifetime fecundity.

We measured male reproductive success (for 100 males total) using the same protocol as for females, but a single LH_M male replaced the single LH_M female in the adult competition vial, and this male was combined with 15 LH_M-bw competitor males and 16 LH_M-bw females (Pischedda and Rice 2012). On Day 14.25, we transferred the LH_M-bw females to individual oviposition vials for 18 h, and male reproductive success was measured 12 days later as the total number of red-eyed offspring (fathered by the LH_M male) produced across all 16 females. This measure of reproductive success includes all offspring sired by each male across these 16 females, including any resulting from matings in the juvenile competition vial. Note that these measures of male and female reproductive success include the intrinsic survival of progeny to adulthood, but not the random offspring mortality

applied to regulate population size (i.e. the culling of eggs at the end of the oviposition stage and the culling of adults at the end of the juvenile competition stage).

(d) Statistical analysis

The experiments measuring reproductive success spanned 10 replicates conducted in succession, and environmental variation across generations caused the average female fecundity to vary. We used the raw data to obtain sex-specific distributions and mean values of reproductive success, but we calculated variance using the residuals from 1-way ANOVAs of reproductive success vs. replicate for each sex. This procedure allowed us to measure the variation in reproductive success that would have occurred in a single generation.

To estimate the distribution of reproductive success after the random culling needed to keep the population size constant (i.e., the fitness measures that correspond to the model of mortality developed by Crow and Morton 1955), the number of offspring produced by each male and female was randomly culled using a binomial random number generator with the arguments N = number of offspring produced and $\text{prob}(\text{success}) = 2/\text{average number of offspring produced by all males or females, respectively}$. 100 simulated culls were performed per individual.

Finally, we calculated bootstrapped 95% confidence intervals (CIs) for all variance/mean ratios and N_e calculations using 10,000 bootstraps. All analyses were completed using JMP Pro 11.

Results

(a) Juvenile fitness distributions

Figure 1 shows the distributions of the number of surviving adult males, females, and both sexes combined from cohorts of 60 eggs ($N = 138$ cohorts; Friberg et al. 2011). On average, 86.1% of eggs survived to the adult stage, and the distribution of the number of surviving individuals (both sexes combined) conforms closely to a binomial distribution (Goodness-of-fit-test, $\chi^2 = 51.77$, $p = 0.70$). There was no difference in the number of surviving males and females (Wilcoxon test of the survival difference, $p = 0.22$), and the distribution of survivors per 60 eggs for each sex conforms to a binomial distribution (Males: Goodness-of-fit-test, $\chi^2 = 24.37$, $p = 0.91$; Females: Goodness-of-fit-test, $\chi^2 = 33.23$, $p = 0.60$). We conclude that egg-to-adult survival is homogeneous between the sexes and that the distribution of survival closely approximates a binomial distribution.

(b) Distributions of adult reproductive success

Figure 2a shows distributions of reproductive success for 100 eggs that survived to become adult males and 100 eggs that survived to become adult females. The distributions are markedly different: males have a modal fitness of 0 offspring, but the lowest fitness value for females was 19 offspring (mode = 57 offspring). The mean reproductive success ($\pm$ SE) of each sex (which were measured in independent experiments) was similar (males: 42.84 ± 3.82 ; females: 45.23 ± 1.13), but, as expected due to stronger sexual selection in males

(Bateman 1948), the variance in male reproductive success far exceeded that of females (by a factor of 13.8; male variance = 1228.86, female variance = 89.03). The variance/mean ratios (σ^2_W/μ_W) were estimated to be 28.69 for male reproductive success and 1.97 for female reproductive success (Table 1), both of which are significantly greater than the variance/mean ratio expected for a Poisson distribution (in which $\sigma^2_W/\mu_W = 1$). The values reported here represent measures of reproductive success that do not include the random mortality that occurs as offspring advance to later stages in the lifecycle of the LH_M population.

(c) Distributions of adult reproductive success after including random offspring mortality to prevent population growth

The fitness values depicted in Figure 2a represent the lifetime offspring production for each individual without the incorporation of offspring mortality. Measures of realized reproductive success will differ from these values due to the random culling of eggs at the start of the juvenile competition vials (i.e. end of the oviposition stage) and the random culling of adult offspring at the start of the adult competition vials (i.e. end of the juvenile competition stage). Figure 2b shows the simulated distributions of reproductive success after this random offspring mortality makes the mean reproductive success of both sexes two offspring. When we incorporated this mortality by applying the correction in equation (2) to the sex-specific means and variances for reproductive success calculated above and shown in Figure 2a, the variance/mean ratios (σ^2_W/μ_W) were reduced from 28.69 (before culling) to 2.29 for males and from 1.97 to 1.04 for females (Table 1); the latter are the values necessary for calculating N_e for males and females. These sex-specific variance/mean ratios are significantly greater than those expected for a Poisson distribution (in which $\sigma^2_W/\mu_W = 1$), although the female distribution is nearly Poisson (bootstrapped 95% confidence interval for $\sigma^2_W/\mu_W = 1.02$ -1.07; Table 1).

(d) Effective sizes of the LH_M population and its different genomic components

We used the male and female variance/mean ratios (σ^2_W/μ_W) calculated for reproductive success that includes random offspring mortality to prevent population growth (reported in section (c) above) to estimate $N_{e(M)}$, $N_{e(F)}$ and $N_{e(A)}$ in the LH_M population ($N = 1,792$, $N_M = 896$, $N_F = 896$) using equations (3) and (4) described above. These values are $N_{e(M)} = 544$, $N_{e(F)} = 877$, and $N_{e(A)} = 1,343$ respectively. Recall that $N_{e(A)} = N_e$ of the whole population. We then used these estimates to calculate N_e for the X and Y sex chromosomes and the cytoplasmic genome using equations (5), (6) and (7), respectively. N_e estimates for these genomic components are $N_{e(X)} = 1093$, $N_{e(Y)} = 272$, and $N_{e(C)} = 439$. The corresponding N_e/N values for all measures are shown in Table 2.

(e) The influence of reproductive success, sexual selection and offspring mortality on N_e

To demonstrate how our results apply to similar stable populations with different fecundities and strengths of sexual selection, we plotted N_e/N as a function of mean reproductive success (before the inclusion of random offspring mortality to prevent population growth) when the variance in male reproductive success is greater than the variance in female reproductive success by differing amounts. The extent to which the variance in male reproductive success exceeds the variance in female reproductive success can be used as an

estimate of the intensity of sexual selection acting in that population (Wade 1979). For simplicity, the variance/mean ratio for females in these simulations is fixed at 1. We calculated the predicted N_e/N ratios for the population as a whole (and the autosomes) using equations (3) and (4) above (Figure 3a). We also calculated the predicted $N_{e(X)}/N_{e(A)}$ ratios using equations (3), (4) and (5) above (Figure 3b).

Discussion

In this study we used an outbred, laboratory-adapted population of *D. melanogaster* to calculate N_e using fitness distributions for males and females. We found equal numbers of surviving males and females when we measured juvenile survival, indicating that our measures of reproductive success will not be affected by a skewed adult sex ratio. The high similarity between the distributions of juvenile survival for males and females (Figure 1) was expected, because the sexes are selected in similar ways during the larval part of the lifecycle when their gender roles are minimally diverged (Chippindale et al. 2001). Although there is heritable variation for juvenile survival in this population (Chippindale et al. 2001), the close fit to a binomial distribution indicates that much of this juvenile mortality is random. In contrast, we observed substantial sexual dimorphism in the distributions of adult reproductive success (Figure 2a). The variance in reproductive success was far higher for males than females (13.8-fold higher), consistent with the strong sexual selection that has been documented in both wild (Markow 1988) and laboratory (Bateman 1948) populations of *D. melanogaster*, including the population used for this study (LH_M; Pischedda and Rice 2012).

Our experiments were designed to mimic the standard culture conditions of our laboratory-adapted population (LH_M) as close as possible, but some minor deviations were necessary that could affect our fitness measures. First, our experiments measuring both male and female reproductive success required females to be held singly in oviposition vials, instead of in groups of multiple females and males (as is standard for this population). Although there is no limitation for oviposition sites in this population, the absence of males during the oviposition stage could remove the potential for additional matings and eliminate harassment that females normally encounter during egg laying. It is unclear how these changes would affect our results, but the oviposition stage only accounts for the final 18 hours of a 2-week lifecycle, so the effects should be minimal. An additional consequence of females being held singly during the oviposition phase is that their offspring encountered lower levels of competition as larvae than usually occurs in this population. This could lower the variance in offspring survival, and cause us to underestimate the variance in reproductive success for both sexes. A second necessary change in our experiments was the use of mutant competitor flies (LH_M-*bw*), made by backcrossing the *bw* mutation into our wild-type LH_M population. Previous work from our lab found no difference in the mean reproductive success of wild-type and mutant *bw* females, but *bw* males had approximately 27% lower reproductive success when in competition with wild-type LH_M males (Stewart et al. 2005). Because our experimental males encountered slightly inferior competitors, our variance for male reproductive success could be an underestimate, and the strength of sexual selection operating in this population could be even stronger than we report here.

With these potential caveats in mind, we can use our sex-specific measures of reproductive success that include random offspring mortality to calculate the N_e of this population. Specifically, N_e is calculated using the variance/mean ratio for males and females. Sexual selection on males increases their variance/mean ratio compared to females, lowering the overall N_e of a population. The strong sexual selection in this population is evident by the sexually dimorphic distributions of reproductive success that do not include offspring mortality (Figure 2a and Table 1). When we incorporated random offspring mortality into these measures of reproductive success, however, the distributions converged dramatically (Figure 2b and Table 1). Thus, offspring mortality scaled the reproductive success of both males and females and caused the apparent strong sexual selection to become far weaker, resulting in an N_e/N ratio of 0.75 (Table 2).

Why would we expect random offspring mortality to so strongly reduce the influence of sexual selection on N_e ? Consider the simplest case where mortality is deterministic, such that the number of offspring produced by each individual is reduced by a factor of $2/\mu_W'$, where μ_W' is the average number of offspring produced per adult before the random deaths of offspring needed to keep the population stable. In general, the mean (μ) of a random variable multiplied by a constant, k , is μk , and the variance (σ^2) is $k^2\sigma^2$. When k is less than one, the variance is more strongly reduced compared to the mean (by a factor of k), and hence the variance/mean ratio is reduced. The same logic applies when offspring numbers are randomly culled by a factor of $2/\mu_W'$ (i.e. mean fitness multiplied by a constant less than 1), so random offspring mortality is expected to reduce the variance/mean ratio of offspring numbers. One possible exception would be if the variance/mean ratio before offspring mortality were less than 1. Although not biologically likely, the addition of random offspring mortality under these conditions would instead increase the variance/mean ratio due to the additional variance associated with this binomial sampling. While this simplified example scales the variance/mean ratio for male and female reproductive success by the same factor (k), it assumes mortality is deterministic rather than stochastic, as occurs in our population. As seen in equation (2) above, the correction for offspring mortality developed by Crow and Morton (1955) scales the variance/mean ratio for males (or the sex experiencing stronger sexual selection) much more strongly than for females.

Our N_e/N ratios for populations with varying fecundities and strengths of sexual selection (Figure 3a) reveal that even very strong sexual selection can have only minimal effects on N_e in stable populations with high rates of random offspring mortality. Offspring mortality thus reduces the potential negative impact of sexual selection on N_e , a finding that may help to explain the persistence of genetic variation for fitness despite strong sexual selection (Kirkpatrick and Ryan 1991). It should be noted that our estimates of N_e are based on single locus neutral theory (Crow and Morton 1955; Lande and Barrowclough 1987), and as a result should be considered an upper bound. Selection, both within and among loci, can further reduce N_e (Robertson 1961; Hill 2007), and these effects are not included in our estimates.

This influence of offspring mortality on N_e is easy to quantify and understand in our laboratory population, but might we expect similar patterns to occur in other systems? Many species, particularly those that produce numerous offspring and/or live in unpredictable

environments, likely experience some degree of random offspring mortality. Although the random culling in our population of *D. melanogaster* is likely more extreme than in other species, we know of at least two studies in natural populations that are consistent with our findings. In the lek-breeding European treefrog (*Hyla arborea*), the ratio of effective breeding size/census breeding size (determined using microsatellite loci) for males (0.44) was only slightly lower than that of females (0.57) despite strong sexual selection (Broquet et al. 2009), a finding that may be attributable to the high pre-reproductive mortality rate in this species (Fiedl and Klump 1997). While this pattern may be less extreme for species with low rates of offspring mortality and higher parental investment in individual offspring, a study in pronghorn (*Antilocapra americana*) found that predation on fawns limited the impact of sexual selection on the population (Byers and Dunn 2012), indicating that a relationship between offspring mortality and the genetic consequences of sexual selection may be widespread.

In addition to influencing the N_e of the whole population, sexual selection can also affect the N_e of various genomic components. For example, the Y chromosome and cytoplasmic genome have the same average number of copies in a population, so any difference in their N_e should be due to sexual selection, which the Y chromosome experiences more strongly. We found that $N_{e(Y)}/N_{e(A)} = 0.20$ and $N_{e(C)}/N_{e(A)} = 0.33$ (Table 2); being restricted to females rather than males increased the N_e of the cytoplasmic genome by a factor of 1.65 compared to the Y chromosome. Similarly, the X chromosome is more strongly sheltered from sexual selection than the autosomes because it spends $2/3^{\text{rds}}$ of its time in females. Thus, under strong sexual selection, the N_e of the X could potentially become larger than the N_e of the autosomes despite having a smaller number of copies (Figure 3b). We found that $N_{e(X)}/N_{e(A)} = 0.81$ (Table 2), indicating that the N_e of the X remained significantly lower than that of the autosomes despite strong sexual selection in this population. Our simulated $N_{e(X)}/N_{e(A)}$ ratios show that sexual selection alone rarely causes the N_e of the X chromosome to be greater than the N_e of the autosomes (Figure 3b).

Our finding that $N_{e(X)}/N_{e(A)}$ is only slightly above its random mating expectation of 0.75 has implications for studies that compare genetic variation between the X and autosomes to interpret the strength of sexual selection operating in a population. For example, Qui et al. (2010) used nucleotide diversity to estimate effective sizes for the autosomes, X and Y chromosomes in *Silene latifolia* and concluded that sexual selection was not operating on males because the X/A diversity ratio was 0.747. Seed mortality in this species is substantial, however (Purington and Schmitt 1995), so the X/A ratio may greatly underestimate the strength of sexual selection (Table 2; Figure 3b). Our results demonstrate that it is difficult to infer how strongly sexual selection is operating in a population using comparisons of X and autosome diversity alone. Although we do not currently have genomic sequence data for this population, it would be valuable to see how our N_e calculations based on fitness variation compare to those obtained using molecular genetic variation.

The substantial offspring mortality in our laboratory-adapted population of *D. melanogaster* created a scaling effect that strongly reduced the influence of sexual selection on N_e , a result that will apply to other high fecundity species. Although offspring mortality is difficult to

measure in many populations, our study demonstrates the importance of this parameter for calculating N_e , and points to a correction that can be used to incorporate random offspring mortality into early offspring counts from stable populations. Studies that measure reproductive success without considering offspring survival may overestimate the genetic consequences of sexual selection and underestimate the effective size of the population.

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Figure 1.

The distributions of juvenile survival (egg-to-adult viability) for (a) males, (b) females, and (c) both sexes combined. Each distribution shows the number of eggs (out of 60 total, including both sexes) that survived to adulthood for 138 total broods.

a. Adult Reproductive Success **b. Adult Reproductive Success including Offspring Mortality** **Figure 2.**

The distributions of (a) reproductive success, and (b) reproductive success after the random offspring mortality needed to prevent population growth. Each distribution is shown for both males (left distributions) and females (right distributions). Reproductive success (a) is measured as the lifetime offspring production for 100 individuals of each sex. Reproductive success including offspring mortality (b) applies random offspring mortality to the distributions in (a) to make the mean reproductive success for both sexes two offspring.

Figure 3.

Simulated values of (a) N_e/N and (b) $N_{e(X)}/N_{e(A)}$ for stable populations (i.e. neither increasing nor decreasing) as a function of mean reproductive success when the variance in male reproductive success exceeds the variance in female reproductive success by differing amounts (i.e. varying the strength of sexual selection). For simplicity, the variance/mean ratio for females in these calculations is fixed at 1. N_e/N and $N_{e(X)}/N_{e(A)}$ ratios are calculated using the random offspring mortality correction developed by Crow and Morton (1955) that prevents the population from growing. The grey horizontal line in (a) indicates the maximum possible value for N_e/N (1), while the grey horizontal line in (b) indicates the expected value of $N_{e(X)}/N_{e(A)}$ based only on differences in copy number between the X and autosomes (0.75).

Table 1

The variance/mean ratios (including bootstrapped 95% confidence intervals) for males and females estimated from distributions of reproductive success with and without the inclusion of the random offspring mortality needed to prevent population growth. The addition of random offspring mortality makes the mean reproductive success for each sex two offspring.

Fitness Component	Variance/Mean (95% CI)	
	Males	Females
Reproductive success	28.68 (20.11-37.68)	1.97 (1.44-2.51)
Reproductive success (including offspring mortality)	2.29 (1.88-2.80)	1.04 (1.02-1.07)

Table 2

N_e and N_e/N estimates (including bootstrapped 95% confidence intervals) for males, females, autosomes (i.e. the population as a whole), the X and Y sex chromosomes and the cytoplasmic genome in our laboratory-adapted population of *D. melanogaster*. Shown for each are the N_e and N_e/N estimates obtained using measures of reproductive success that incorporate the random offspring mortality needed to prevent population growth ($N=1792$, $N_{\text{males}} = 896$, $N_{\text{females}} = 896$). For males and females, these ratios were obtained using $N_{e(\text{sex})}/N_{(\text{sex})}$. Also included are the copy numbers for the sex chromosomes and cytoplasmic genomes relative to the autosomes.

Census Population	Copy Number (relative to autosomes)	N_e (95% CI)	N_e/N (95% CI)
Males	-	544 (472-622)	0.607 (0.527-0.694)
Females	-	877 (896-887)	0.979 (0.967-0.990)
Autosomes/Population	1	1344 (1227-1456)	0.750 (0.685-0.813)
X Chromosome	0.75	1093 (1023-1158)	0.61 (0.571-0.646)
Y Chromosome	0.25	272 (236-311)	0.152 (0.132-0.174)
Cytoplasm	0.25	439 (433-444)	0.245 (0.242-0.248)