# Inbreeding Depression and Inferred Deleterious-Mutation Parameters in Daphnia

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Manuscript received October 16, 1996
Accepted for publication May 19, 1997

ABSTRACT

DENG and LYNCH recently proposed a method for estimating deleterious genomic mutation parameters from changes in the mean and genetic variance of fitness traits upon inbreeding in outcrossing populations. Such observations are readily acquired in cyclical parthenogens. Selfing and life-table experiments were performed for two such Daphnia populations. We observed a significant inbreeding depression and an increase of genetic variance for all traits analyzed. DENG and LYNCH's original procedures were extended to estimate genomic mutation rate ($U$), mean dominance coefficient ($\bar{h}$), mean selection coefficient ($\bar{s}$), and scaled genomic mutational variance ($V_m/V_e$). On average, $\hat{U}$, $\hat{\bar{h}}$, $\hat{\bar{s}}$ and $\hat{V}_m/V_e$ ($\hat{}$ indicates an estimate) are 0.74, 0.30, 0.14 and 4.6E-4, respectively. For the true values, the $\hat{U}$ and $\hat{\bar{h}}$ are lower bounds, and $\hat{\bar{s}}$ and $\hat{V}_m/V_e$ upper bounds. The present $\hat{U}$, $\hat{\bar{h}}$ and $\hat{V}_m/V_e$ are in general concordance with earlier results. The discrepancy between the present $\hat{\bar{s}}$ and that from mutation-accumulation experiments in Drosophila (~0.04) is discussed. It is shown that different reproductive modes do not affect gene frequency at mutation-selection equilibrium if mutational effects on fitness are multiplicative and not completely recessive.

INBREEDING depression has been documented in almost all organisms that have been examined (CHARLESWORTH and CHARLESWORTH 1987; FALCONER 1989). The magnitude of inbreeding depression has many implications in modern evolutionary theory, such as the evolution of self-incompatibility systems in monoecious plants (LANDE and SCHEMSKE 1985; SCHEMSKE and LANDE 1985; CHARLESWORTH and CHARLESWORTH 1987) and the evolution of dispersal mechanisms for inbreeding avoidance in animals (SHIELDS 1982). While it is well known that inbreeding can cause a change of both mean genotypic value and genetic variance (CROW and KIMURA 1970; FALCONER 1989), most empirical and theoretical work on inbreeding related subjects has been concentrated on the change of the mean (*c.f.* LANDE and SCHEMSKE 1985; SCHEMSKE and LANDE 1985; CHARLESWORTH and CHARLESWORTH 1987; FALCONER 1989; CHARLESWORTH *et al.* 1990). Study of the change of genetic variance upon inbreeding may provide some valuable information on evolutionary processes (ROBERTSON 1952; COCKERHAM 1984a,b; GOODNIGHT 1987, 1988; WILLIS and ORR 1993; SCHULTZ and WILLIS 1995), such as an inflated additive genetic variance upon population bottlenecks. Moreover, joint information on the response of mean genotypic values and genetic variances to inbreeding can be used to estimate spontaneous deleterious genomic mutation parameters (DENG and LYNCH 1996a), such as the genomic mutation rate to mildly deleterious alleles ($U$).

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Estimates of $U$ are crucial to testing theories for the evolution of sex (MULLER 1964; KONDRASHOV 1985, 1988; CHARLESWORTH 1990), mate choice (KONDRASHOV 1988; CHARLESWORTH and CHARLESWORTH 1987; KIRKPATRICK and RYAN 1991), outbreeding mechanisms (CHARLESWORTH and CHARLESWORTH 1987), diploidy (KONDRASHOV and CROW 1991), and the accelerated extinction rate of small populations (LYNCH and GABRIEL 1990; LYNCH *et al.* 1993, 1995a,b). However, few estimates are available (CROW and SIMMONS 1983; KONDRASHOV 1988; CROW 1993). A direct estimation approach, the traditional mutation-accumulation experiment (BATEMAN 1959; MUKAI *et al.* 1972), takes extensive time and labor and is only feasible for asexual organisms, special sexual organisms (such as Drosophila where special chromosomal constructs are available), and artificially constructed purely inbred lines. An indirect estimation procedure in highly selfing plants makes use of inbreeding depression data (CHARLESWORTH *et al.* 1990), but it depends on an unknown mean dominance coefficient ($\bar{h}$) of deleterious mutations. Estimation of $\bar{h}$ requires some more assumptions (COMSTOCK and ROBINSON 1948; HAYMAN 1954; MUKAI *et al.* 1972; JOHNSTON and SCHOEN 1995; DENG and LYNCH 1996a). Even then, the estimate is biased, and weighted by selection coefficients of individual mutant alleles. Estimation of other parameters of spontaneous deleterious genomic mutations, such as the mean selection coefficient ($\bar{s}$) and the genomic mutation variance scaled by environmental variance ($V_m/V_e$), is also important for testing different evolutionary theories. For example, estimates of $\bar{h}$ and $\bar{s}$ are important for

testing the theories of the evolutionary transition from haploidy to diploidy (PERROT *et al.* 1991) and of the role of deleterious mutations in extinction of small populations (LANDE 1994; LYNCH *et al.* 1995a). Stimulated by the work of CHARLESWORTH *et al.* (1990), DENG and LYNCH (1996a) developed a methodology, which uses the data (changes of the mean and genetic variance for fitness traits) that can be acquired from inbreeding/outbreeding in outcrossing/highly selfing populations, to estimate not only $U$, but also $\bar{h}$, $\bar{s}$, and $V_m/V_e$.

In regularly outcrossing animals, data on the change of genetic variance for fitness traits upon inbreeding are not readily come by. Since genotypes normally cannot be cloned easily, the total genetic variance cannot be estimated without bias (FALCONER 1989). An unbiased estimate of the total genetic variance requires that clones of the genotypes be available and distributed randomly across the experimental environment, so that there will be no common environmental effects for members of the same genotype and the environmental variation will be clearly separated from the total genetic variance in analyses of variance (ANOVA). In cyclical parthenogens, genotypes can be preserved and replicated (forming a clone) by asexual reproduction, and inbred progeny can be constructed by mating relatives (*e.g.*, mating among clonal members is genetically equivalent to selfing). Thus, outcrossed and inbred genotypes can be assayed side by side in one controlled environment, with each having multiple replicates. Under such a situation, the change of the genetic parameters across generations will not be confounded by temporal environmental change. Performing one-way ANOVA, with clonal genotypes as main effects and clonal replicates for genotypes as random effects, provides unbiased estimates for the total genetic variance for quantitative traits. Thus, cyclical parthenogens are quite suitable for the application of DENG and LYNCH's (1996a) technique.

In this study, inbreeding and life-table experiments were performed on populations of two cyclically parthenogenetic species of the freshwater cladoceran, *Daphnia arenata* and *D. pulicaria*. Changes of the mean and genetic variance were estimated and used to infer deleterious genomic mutation parameters by an extension of DENG and LYNCH's (1996a) procedure.

## MATERIALS AND METHODS

**Study organism:** In nature, the life cycle of cyclically parthenogenetic Daphnia species consists of several consecutive generations of parthenogenetic reproduction followed by a bout of sexual reproduction, during which males and sexual females are produced and mate randomly (LYNCH 1983a,b; HEBERT 1987). Normally, the population size is effectively infinitely large (LYNCH 1983a,b). In the laboratory, parthenogenetic reproduction can be maintained indefinitely as long as environmental conditions remain favorable. During parthenogenetic reproduction, genotypes are faithfully replicated barring new mutation (HEBERT 1987), which makes it possible to estimate the total genetic variance for any quantitative trait by an appropriate experimental design (LYNCH 1985; LYNCH and DENG 1994). Sexual reproduction can be induced reliably in the laboratory, and the resultant resting eggs can be hatched relatively easily (INNES 1989; DE MEESTER 1993; LYNCH and DENG 1994; DENG 1995, 1996, 1997; DENG and LYNCH 1996b). Generation time is ~2 weeks at 20° (LYNCH and ENNIS 1983).

**Study populations and production of selfed progeny:** The experimental populations are from Amazon Park (*D. arenata*) in Eugene, OR, and Dorena Reservoir (*D. pulicaria*) in Cottage Grove, OR. Electrophoretic studies showed that the two populations reproduce by cyclical parthenogenesis with mating within each population being effectively random (LYNCH and DENG 1994; DENG and LYNCH 1996b). Detailed experimental procedures of sampling populations, cloning and selfing genotypes, determining species identity, and hatching selfed resting eggs have been published in LYNCH and DENG (1994), and DENG and LYNCH (1996b). Briefly, populations were sampled at the end of their growing season, when there were still millions of individuals. Females sampled from the field were isolated into individual beakers containing ~200 ml of filtered and aged water from the populations' source habitats and fed with the green alga Scenedesmus. The species identity was determined by morphological (BROOKS 1957; BRANDLOVA 1972) and biochemical (HEBERT *et al.* 1988, 1989; LYNCH *et al.* 1989) criteria. Over a period of 2 months, the isolated females reproduced asexually, forming cohorts of genetically identical individuals. During this period, most clones also reproduced sexually; *i.e.*, males were produced ameiotically, and some asexual females switched to sexual reproduction by producing sexual eggs meiotically. Since males and females in each beaker are genetically identical, mating among them is genetically equivalent to selfing. The resultant sexually produced eggs are in a diapausing form (ephippia) and hatched by taking them through light/warm and dark/cold cycles (LYNCH and DENG 1994; DENG 1995; DENG and LYNCH 1995b). The hatched selfed individuals were then expanded clonally.

**Life-table experiments:** Two life-table experiments were performed, one for each population, with 30 random outcrossed parental clones and 30 random selfed offspring clones (each from 30 different random parental clones) being used for each population. Each genotype was replicated three times and all were acclimated to the experimental conditions for two generations before any measurement, ensuring that maternal and grand-maternal effects do not contribute to the among-clone variance in the final analysis (LYNCH 1985). All clonal replicates were randomly distributed in the experimental setting, so that the common micro-environmental effects would not contribute to the resemblance of the clonal replicates of each genotype, ensuring that genetic variance can be unbiasely estimated. Each clonal replicate was maintained in 100 ml water (aged for at least 1 month and filtered before use) from the populations' source habitats, with ~300,000 cells of the green alga Scenedesmus per ml. For the Amazon population, the experiment was conducted at 20° (a typical daytime temperature during the growing season in the population's source habitat, a seasonal pond), 12 hr:12 hr light-dark photoperiod (typical during this population's growing season), and the culture water was replenished every day. For the Dorena population, the experiment was conducted at 10° (a typical temperature during the growing season in the population's source habitat, bottom of a permanent lake), 16 hr: 8 hr light-dark photoperiod (typical during this population's growing season), and the culture water was changed every other day (due to the slow development of Daphnia in this temperature). Starting from the third generation, newborn individuals were measured daily (Amazon population)

or every other day (Dorena population), from birth to the second (Amazon population) or the third (Dorena population) adult instar, to obtain data for different life-history traits, such as instar-specific body size, age at release of first clutch, growth rates, etc.

**Data analysis:** The final data were subject to one-way ANOVA. The among-clone variance provides an unbiased estimate for the total genetic variance among the clones. The estimates of the standard errors for the genetic variance were obtained by the Taylor expansion approximation method (LYNCH 1985; DENG 1996).

The estimation procedure developed by DENG and LYNCH (1996a) cannot be applied directly to these data, as the original procedure requires measurement of the genetic variance among the *mean of the selfed families*, which are formed by multiple selfed genotypes from each outcrossed parent. The expected value of this is expressed by Equations 1d and 6d under constant and variable mutation effects, respectively, in DENG and LYNCH (1996a). This genetic variance can be estimated by the among selfed-family variance component in a nested ANOVA, with clonal replicates nested within selfed clones that are nested within each selfed family, or by the among selfed-family variance component in a one-way ANOVA, with selfed clones (without clonal replicates) within each selfed families as random effects and selfed families as main effects. However, with one selfed offspring genotype available from each outcrossed parent as in the present study, only the total genetic variance (the sum of the genetic variance among the means of the selfed families and the genetic variance among the selfed genotypes within selfed families) in the selfed offspring generation is estimable. Therefore, we extended DENG and LYNCH's (1996a) original procedure to accommodate the current situation where only total genetic variance in the selfed offspring generation is available (APPENDIX A). The expression for this total genetic variance is given by Equation A1 in APPENDIX A. The statistical properties (bias and sampling errors) of the estimation procedure under a range of biological situations, such as variable mutation effects, variable mutation rate for mutations of different effects and epistatic mutation effects, are investigated by computer simulations (APPENDIX A):

Fecundity is an important fitness component, believed to be under directional natural selection to increase. Thus, the genotypic means ($W$) and genetic variances ($V_g$) for fecudidty in the selfed offspring generation ($s$) and the outcrossed parental generation ($p$) were used to infer $U$, $\bar{h}$, $\bar{s}$, and $V_m/V_e$ by Equations A4–5 (Appendix A).

## RESULTS

**Genetic consequences of inbreeding:** For the Dorena *D. pulicaria* population, selfing resulted in a high magnitude of inbreeding depression for all traits analyzed (Tables 1 and 2). Survivorship to maturity (indicated by the initiation of reproduction) decreased from 0.97 (0.03) to 0.79 (0.06) (throughout, unless otherwise specified, the number in parentheses indicates 1 SE). Egg survivorship for the first clutch declined from 0.23 (0.04) to 0.20 (0.05), and for the second clutch from 0.55 (0.03) to 0.41 (0.07). Average clutch size of the first three clutches declined by 2.3 eggs (columns 2 and 4 in Table 1). In units of the phenotypic standard deviations in the parental generation, changes (in absolute values) as high as 0.65 (for age at maturity), and as low as 0.34 (adult growth rate) were observed (column 2 in Table 2), with an average of 0.52. From the data in columns 2 and 3 in Table 2, it can be seen that one generation of selfing reduced body size at birth by 0.030 mm, body size at maturity by 0.067 mm, juvenile growth rate by 0.0051 ln(mm)/day, and adult growth rate by 0.0014 ln(mm)/day, and increased age at maturity by 0.99 days.

**TABLE 1**

**Summary of the mean ($W$) and genetic variance ($V_g$) for fitness traits for the selfed progeny generation ($s$) and outcrossed parental generation ($p$)**

| | Fitness traits | | | |
|---|---|---|---|---|
| | W ($s$) | $V_{g(s)}$ | W ($p$) | $V_{g(p)}$ |
| Dorena *D. pulicaria* | | | | |
| 1st clutch size | 7.01 | 5.84 | 8.27 | 3.92 |
| | (0.54) | (2.30) | (0.40) | (1.24) |
| 2nd clutch size | 10.44 | 20.85 | 13.19 | 15.13 |
| | (0.97) | (7.35) | (0.68) | (3.58) |
| 3rd clutch size | 13.46 | 30.95 | 16.25 | 24.72 |
| | (1.23) | (11.85) | (0.77) | (4.62) |
| Amazon *D. arenata* | | | | |
| 1st clutch size | 4.85 | 1.49 | 8.00 | 0.99 |
| | (0.43) | (1.05) | (0.42) | (1.21) |
| 2nd clutch size | 12.76 | 5.19 | 16.91 | 1.46 |
| | (1.38) | (11.56) | (0.79) | (4.69) |

The numbers within parentheses indicate 1 SE.

For the Amazon *D. arenata* population, selfing resulted in substantial inbreeding depression (Tables 1 and 2). Egg survivorship of the first clutch declined from 0.81 (0.14) to 0.43 (0.07), and survivorship to maturity from 0.67 (0.07) to 0.38 (0.07). Average clutch size of the first two clutches decreased by 3.65 eggs (columns 2 and 4 in Table 1). In absolute values, changes of other life-history characters ranged from 0.43 to 1.24 in units of parental phenotypic standard deviations (Table 2), with an average of 0.79. From the data in columns 2 and 3 in Table 2, it is seen that one generation of selfing reduced body size at birth by ~0.015 mm and body size at maturity by ~0.040 m, and increased age at maturity by ~3.3 days.

The results for the change of genetic variance upon selfing in both populations were not as conclusive as those for the change of the means (Table 1). The genetic variances uniformly increased upon selfing, though none is statistically significant.

**Estimation of $U$, $\bar{h}$, $\bar{s}$ and $V_m/V_e$:** Estimates for $U$, $\bar{h}$, $\bar{s}$ and $V_m/V_e$ are quite consistent within each population but differ somewhat between the two populations, especially for $\hat{s}$ (Table 3). Averaging over the first three clutches, $\hat{U}$, $\hat{\bar{h}}$, $\hat{\bar{s}}$ and $\hat{V}_m/V_e$ were 0.99, 0.36, 0.21 and 8.9E-4, respectively, for the Dorena *D. pulicaria* population, and 0.69, 0.23, 0.07, 3.4E-5, respectively, in the Amazon *D. arenata* population. Averaging over the two populations, $\hat{U}$, $\hat{\bar{h}}$, $\hat{\bar{s}}$ and $\hat{V}_m/V_e$ were 0.74, 0.30, 0.14, and 4.6E-4, respectively.

**TABLE 2**

**Magnitude of the inbreeding depression resulting from selfing**

| Life-history trait | $[g(s) - g(p)]^a$ | Mean[b] |
|---|---|---|
| Dorena *D. pulicaria* | | |
| Instar-specific body size | −0.64 (0.20) | |
| Body size at birth | −0.62 (0.25) | 0.654 (0.046) |
| Body size at maturity | −0.38 (0.23) | 1.563 (0.177) |
| Juvenile growth rate | −0.46 (0.22) | 0.070 (0.011) |
| Adult growth rate | −0.34 (0.22) | 0.013 (0.004) |
| Age at maturity | 0.65 (0.23) | 11.691 (1.52) |
| Amazon *D. arenata* | | |
| Instar specific body size | −1.24 (0.19) | |
| Body size at birth | −0.44 (0.61) | 0.555 (0.032) |
| Body size at maturity | −0.43 (0.21) | 1.328 (0.104) |
| Juvenile growth rate | −0.82 (0.30) | 0.117 (0.028) |
| Age at maturity | 1.24 (0.15) | 12.28 (2.54) |
| Duration of 1st adult instar | 0.58 (0.37) | 3.55 (0.48) |

*g(s)* and *g(p)* are the mean genotypic values of the selfed progeny generation and of the outcrossed parental generation, respectively, scaled by the phenotypic SD in the parental generation. SE indicates one associated standard error. SE and SD values are in parentheses. The mean and phenotypic standard deviations (SD) for several characters for the parental generation are given in column 3 as reference to provide indications of the absolute magnitude of changes in selfed progeny. For instar-specific body size; separate analyses were performed for the first eight (Dorena *D. pulicaria*) or seven (Amazon *D. arenata*) instars, and final results were averaged; body size at birth is taken to be the mean offspring size for the third (Dorena *D. pulicaria*) or second (Amazon *D. arenata*) adult instar. Body size is measured in mm, adult instar duration and age at maturity are measured in days. The juvenile growth rate is equal to [ln(body size at maturity) − ln(body size at birth)]/(age at maturity), the adult growth rate = [ln(body size of 3rd adult instar) − ln(body size at 1st adult instar)]/(time between the above adult instars).

[a] SE values are in parentheses.

[b] SD values are in parentheses.

The consistency of the estimates within each population is largely due to the high total genetic correlations among the fecundities of different clutches, ($R_G(C_i,C_j)$ = total genetic correlation between the $i$th and $j$th clutch sizes). Using a program supplied by K. SPITZE, we estimated that $R_G(C_1,C_2) = 0.67$, $R_G(C_2,C_3) = 0.91$, $R_G(C_1,C_3) = 0.78$ for the Dorena *D. pulicaria* population, and $R_G(C_1,C_2) = 0.53$ for the Amazon *D. arenata* population, which are in close agreement with previous estimates for different populations or species of Daphnia (SPITZE *et al.*, 1991; LYNCH and SPITZE 1994). Due to the high interdependency of within population estimates as revealed by the high genetic correlations, the above overall average of estimates were computed over the two populations' averages rather than over the estimates for the five individual clutches of the two populations.

Due to the complex nonlinear functional relationship of the mutation parameters with the observed quantities in both generations (Equations A4 and A5), simple analytical approximations for the sampling variances of the mutation parameters are not obtainable. Thus, bootstrapping analyses were attempted. However, due to the small sample size, the genetic variances are very sensitive to the resampling of the original data. For most traits, 1000 bootstrapping analyses could not be carried through, and often large sampling errors were found. To obtain estimations with reasonably small sampling error, larger sample sizes are needed (DENG and LYNCH 1996a; APPENDIX A). Simulation results (APPENDIX A) suggest that 200 clones would result in much better estimates with reasonably small sampling errors.

**TABLE 3**

**Estimates for $U$, $\bar{h}$, $\bar{s}$ and $V_m/V_e$**

| Fitness trait | $\hat{U}$ | $\hat{\bar{h}}$ | $\hat{\bar{s}}$ | $\hat{V}_m/V_e$ |
|---|---|---|---|---|
| Dorena *D. pulicaria* | | | | |
| 1st clutch size | 0.79 | 0.35 | 0.20 | 1.2E-3 |
| 2nd clutch size | 1.04 | 0.35 | 0.23 | 8.2E-4 |
| 3rd clutch size | 1.15 | 0.38 | 0.21 | 6.5E-4 |
| Average | 0.99 | 0.36 | 0.21 | 8.9E-4 |
| Amazon *D. arenata* | | | | |
| 1st clutch size | 1.00 | 0.25 | 0.06 | 5.3E-5 |
| 2nd clutch size | 0.38 | 0.20 | 0.07 | 1.4E-5 |
| Average | 0.69 | 0.23 | 0.07 | 3.4E-5 |

## DISCUSSION

The present study not only adds inbreeding data from cyclical parthenogens to the existent abundant inbreeding literature in purely sexual organisms (CHARLESWORTH and CHARLESWORTH 1987; FALCONER 1989), but also documents the change of genetic variance brought about by selfing, the study of which is rare. Particularly, the results were used to infer the genomic mutation parameters ($U$, $\bar{h}$, $\bar{s}$ and $V_m/V_e$), all of which are normally difficult to obtain by experimental means.

The magnitude of inbreeding depression is in agreement in the two study populations. In the Dorena *D. pulicaria* population, we observed declines of 19% in survivorship to maturity, 15% in egg survivorship of the first clutch, 24% in egg survivorship of the second clutch. In the Amazon *D. arenata* population, we observed declines of 43% in survivorship to maturity, 47% in egg survivorship of the first clutch. These quantities are also in agreement with previous selfing experiments in *D. laevis* (BANTA 1939), *D. obtusa* (INNES 1989) and *D. magna* (DE MEESTER 1993), all of which found a decrease of survivorship to maturity or egg survivorship of ~20–50%. DE MEESTER (1993) invoked the observed high inbreeding depression to explain that inbreeding in natural Daphnia populations is very rare. This idea is consistent with the general observations from ecological genetics studies that mating is usually effectively random in Daphnia natural populations (LYNCH 1983a; HEBERT 1987; LYNCH and SPITZE 1994).

In units of phenotypic standard deviations in the parental generations, the Dorena *D. pulicaria* population had about the same magnitude of inbreeding depression as the Amazon *D. arenata* population (column 2 in Table 2). On average, a shift of 0.52 and 0.79 phenotypic standard deviations resulted from a generation of selfing in the Dorena *D. pulicaria* population and the Amazon *D. arenata* population, respectively. In purely sexual organisms, averaging over 13 traits in six organisms (ranging from humans to maize) (Table 14.1, FALCONER 1989), one generation of selfing results in a shift of the mean of ~1.1 phenotypic SD in regularly outcrossing populations (direct or extrapolated results). Thus, the magnitude of inbreeding depression for the two study populations of cyclical parthenogens is comparable to that for purely sexual species.

Due to the potential variable mutation effects and epistatic effects (DENG and LYNCH 1996a; APPENDIX A), the genomic mutation parameters estimated here are lower bounds in the case of $\hat{U}$, $\hat{\bar{h}}$, and upper bounds in the case of $\hat{\bar{s}}$ and $\hat{V}_m/V_e$. However, the estimates are not biased by the peculiar life cycle of cyclical parthenogens (APPENDIX B). The $\hat{U}$'s and $\hat{\bar{h}}$'s are in close agreement with earlier results in Drosophila (MUKAI *et al.* 1972) and in natural plant populations (JOHNSTON and SCHOEN 1995). The present study estimated that for fecundity, $\hat{U}$ on average is at least 0.99 in the Dorena *D. pulicaria* population and 0.69 in the Amazon *D. arenata* population. In Drosophila, $U$ is estimated to be at least 0.84 for viability by mutation-accumulation experiments (MUKAI *et al.* 1972). An indirect estimation (JOHNSTON and SCHOEN 1995) also gave some compatible results in regularly selfing plants in nature, where the lower bound for $U$ ranged from 0.24 to 0.87, with an average of 0.57. The estimates for $\bar{h}$, which on average is at least 0.36 for the Dorena *D. pulicaria* population, and 0.23 for the Amazon *D. arenata* population, are comparable with that in Drosophila (MUKAI *et al.* 1972), where it is estimated to be about 0.30 for mildly deleterious genomic mutations. JOHNSTON and SCHOEN (1995) also estimated $\bar{h}$ for mildly deleterious mutations in four populations of two obligate selfing plant species by crossing experiments, obtaining $\hat{\bar{h}}$ for total fitness ranging from 0.07 to 0.35, with an average of 0.22. The estimates for the upper bound of $V_m/V_e$, which are 8.9E-4 for the Dorena *D. pulicaria* population and 3.5E-5 for the Amazon *D. arenata* population, are in the lower range estimated for a diversity of organisms (LYNCH 1988), which is approximately from 0.0001 to 0.05; in particular, they are also within the lower range estimated for diverse life-history traits in Daphnia by a direct mutation accumulation experiments (LYNCH 1985), which is ~0.0008–0.0036.

Although our estimates of $\bar{s}$ differ to a large extent from earlier results in Drosophila from mutation-accumulation experiments (MUKAI *et al.* 1972), they are not inconsistent, since all of them are just upper bounds for the true $\bar{s}$. Even within the present study, there was relatively large variation for the $\bar{s}$ estimates. For example, the smallest upper bound estimated for $\bar{s}$ was 0.06 (first clutch size in the Amazon *D. arenata*), while the largest was 0.23 (second clutch size in the Dorena *D. pulicaria*). On average, the upper bound of $\bar{s}$ is 0.21 for the Dorena *D. pulicaria* population, and 0.07 for the Amazon *D. arenata* population. In Drosophila, the upper bound of $\bar{s}$ estimated by mutation-accumulation experiments is about 0.04 for viability (MUKAI *et al.* 1972). One thing of note is that the upper bound of $\bar{s}$ estimated from these studies is only for mildly deleterious mutations (lethal mutations excluded) on the second chromosome, whereas $\bar{s}$ estimated in the present study using DENG and LYNCH's technique is for all genomic deleterious mutations (including lethal mutations). Thus, the higher upper bounds of $\bar{s}$ estimated here may not be so surprising. In fact, CHARLESWORTH and CHARLESWORTH (1987) summarized earlier inbreeding data and concluded that a substantial proportion of inbreeding depression (~50%) is attributable to lethal mutations. In any case, the parameters for genomic deleterious mutations are not expected to be identical for different organisms, or for different fitness traits (even of the same organism), as recently demonstrated by KIBOTA and LYNCH (1996).

It should be noted that all of the assumptions used by our estimation approach are essentially the same as those used by CHARLESWORTH *et al.* (1990); some are the same as those employed in the traditional mutation-accumulation approach (BATEMAN 1959; MUKAI *et al.* 1972; HOULE *et al.* 1992). For example, the Bateman-Mukai technique assumes a Poisson distribution of mutation occurrence in the genome and that mutations at different loci underlying the trait are in gamatic phase equilibrium. However, the labor and the time to perform traditional mutation-accumulation experiments greatly exceed that necessary for the application of our approach using populations at mutation-selection equilibrium. The technique of CHARLESWORTH *et al.* (1990) may yield an estimate for $U$, but requires a *priori* knowledge of $\bar{h}$, whose estimation requires more experiments and assumptions (MUKAI *et al.* 1972; JOHNSTON and SCHOEN 1995; DENG and LYNCH 1996a).

Stimulated by the ideas of MORTON *et al.* (1956) and CHARLESWORTH *et al.* (1990) that mutation parameters may be estimated from natural populations at mutation-selection equilibrium, we developed more powerful procedures in DENG and LYNCH (1996a) and extended it here. The extension we made here is likely to be even more powerful and useful than DENG and LYNCH's (1996a) original procedure. Without the requirement of obtaining multiple selfed genotypes from each outcrossed parent, estimation of total genetic variance in the selfed generation should be easier and probably more accurate than estimation of the genetic variance among the mean of selfed families. The experiments

here were performed by the authors with some assistance in a total of ~12 months. Larger scale experiments such as those employing ~200 clones should be carried out to achieve estimates with relatively small sampling errors. This kind of experiment is being planned and, once initiated, should be accomplished within about the same time frame as our present study.

It should be noted that selfing is not essential with our approach, though cloning of genotypes is done to separate environmental variance from genetic variance. For the situation of full-sib mating, an approximate formulation is given by Equation 14 of DENG and LYNCH (1996a). We (H-W. DENG and M. LYNCH, unpublished results) have worked out the exact formulation for experimental settings of any degree of inbreeding (such as full sib and half sib mating etc.), so that as long as genotypes can be cloned, our approach can be applied. Many evolutionary theories evoke deleterious mutation as an essential explanation for some fundamental biological phenomena. Now it may be possible to test those theories by estimating mutation parameters in a wider variety of taxa through indirect inference from populations at mutation-selection equilibrium.

We thank Z. BANKS, S-K. LEE, Q. TIAN and S-L. YU for laboratory assistance, and K. SPITZE for supplying a program to compute the total genetic correlations by MANOVA analyses. We also thank Dr. A. CLARK and two anonymous reviewers for insightful and useful comments, which helped to improve our paper. The work was supported by National Science Foundation grant 89-11038 and Public Health Service grant R01 GM-36827-01 to M. LYNCH. While preparing the manuscript, H-W.D. was supported as a postdoctoral fellow by a First Award from National Institutes of Health to Dr. Y-X. FU.

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Communicating editor: A. G. CLARK

## APPENDIX A

DENG and LYNCH's (1996a) estimation procedures are based on a number of assumptions: the population is very large and at mutation-selection equilibrium; the fitness function is multiplicative; mutations at different loci are in gamatic phase equilibrium; mutations on different loci have constant effects $s$ (selection coefficient) and $h$ (dominance coefficient); and the number of mutations per genome ($n$) is Poisson distributed with probability density function $p(n) = \bar{n}^n e^{-\bar{n}}/n!$, where $\bar{n}$ is the mean number of deleterious mutations per genome. Under these assumptions, the total genetic variance ($V_g(s)$) in the offspring generation resulting from one generation of selfing parental genotypes is

$$V_g(s) = W_{\max}^2 \sum_{n=0}^{\infty} \left(\frac{1}{4} + \frac{(1-hs)^2}{2} + \frac{(1-s)^2}{4}\right)^n \times p(n) - W^2(s) = W^2(s)\left[\exp\left(\frac{Uhs}{2} + \frac{Us}{4h}\right) - 1\right], \quad \text{(A1)}$$

where $W_{\max}$ and $W(s)$ are, respectively, the expected fitness of a mutation-free genotype in an experimental setting and the mean fitness of the selfed offspring generation.

Equation A1 is derived as follows. Let $w(s)$ represent the fitness of a genotype from the selfed offspring generation, then $V_g(s) = E(w^2(s)) - W^2(s)$, and $E(w^2(s)) = \sum_{n=0}^{\infty} E(w^2(s)/n)p(n)$, where $E(w^2(s)/n)$ is the expectation of $w^2(s)$ conditional on the parent having mutations at $n$ loci. Letting $x_i$ denote the fitness of the $i$th locus (which is heterozygous in the outcrossed parent) in a selfed progeny, under the assumptions of multiplicative fitness and gamatic phase equilibrium,

$$E(w^2(s)/n) = E(\textstyle\prod_{i=0}^{n} x_i^2) = \prod_{i=0}^{n} E(x_i^2).$$

For each parental locus that is heterozygous for mutations, the selfed progeny are expected to be heterozygous or homozygous for the deleterious alleles with probabilities $^1/_2$ and $^1/_4$ respectively, hence

$$E(x_i^2) = \frac{1}{4} + \frac{(1-hs)^2}{2} + \frac{(1-s)^2}{4},$$

which is independent of the $i$th locus under the assumption of constant mutation effects across loci. Therefore, we have

$$E(w^2) = \sum_{n=0}^{\infty} \left(\frac{1}{4} + \frac{(1-hs)^2}{2} + \frac{(1-s)^2}{4}\right)^n p(n). \quad \text{(A2)}$$

$W_{\max}$ is just a scaling factor so that fitness measurement can be on any scale instead of just from 0.0 to 1.0, and also so that mean environmental effects of experiments do not influence estimation. From Equation 1c of DENG and LYNCH (1996a), we have

$$W(s) = W_{\max} \exp\left(-\frac{U(2h+1)}{4h}\right). \quad \text{(A3)}$$

By (A2) and (A3) and making use of the relationship $\bar{n} = U/(hs)$ (DENG and LYNCH 1996a) for large populations at mutation-selection equilibrium, Equation A1 can be obtained.

Solving Equation A1 and Equations 1a–c of DENG and LYNCH (1996a), we have

$$\hat{h} = \frac{1}{2\sqrt{\hat{z}/\hat{x} - 1/2}} \quad \text{(A4a)}$$

$$\hat{U} = \frac{4\hat{h}}{2\hat{h} - 1} \quad \text{(A4b)}$$

$$\hat{s} = \frac{\hat{x}}{\hat{U}\hat{h}} \quad \text{(A4c)}$$

$$\frac{\hat{V}_m}{V_e} = \frac{\hat{U}^*(\hat{h}\hat{s})^2}{\hat{V}_e}, \quad \text{(A4d)}$$

where the circumflex ( $\hat{}$ ) indicates an empirical estimate. In the present study, $V_e$ is estimated by the average of the within-clone variances in the separate one-way ANOVA analyses in the outcrossed parent and selfed progeny generations, and

$$\hat{x} = \ln\left(\frac{V_g(p)}{W^2(p)} + 1\right) \quad \text{(A5a)}$$

$$\hat{y} = \ln\left(\frac{W(s)}{W(p)}\right) \qquad \text{(A5b)}$$

$$\hat{z} = \ln\left(\frac{V_g(s)}{W^2(s)} + 1\right), \qquad \text{(A5c)}$$

where $W(s)$ and $W(p)$ are the genotypic means, $V_g(s)$ and $V_g(p)$ are the total genetic variances, in the selfed offspring ($s$) and outcrossed parental ($p$) generations, respectively.

The statistical properties (sampling variance and bias) and robustness of the above analytical results were investigated by computer simulations. Since the simulation procedures are already detailed in DENG and LYNCH (1996a), we do not repeat them here, except to note that the total genetic variance in the selfed progeny (instead of the genetic variance among the selfed family means) is used. Simulations were performed for a most likely parameter set in Drosophila (MUKAI *et al.*, 1972; HOULE *et al.*, 1992; LYNCH *et al.*, 1995b), $U = 1.5$, $\bar{h} = 0.36$, $\bar{s} = 0.03$, $V_m = 1.75\text{E-}4$ (when mutation effects are constant). Simulations were performed by assuming that all genotypic values were known without error. Violation of this assumption, which is usually the case in practice, will unlikely bias the estimation as shown in DENG and LYNCH (1996a), but will inflate the sampling variance of estimates. In each simulation, unless otherwise specified, 200 outcrossed parents were sampled from a population at mutation-selection equilibrium, and from each of which, one selfed offspring genotype was randomly determined as in DENG and LYNCH (1996a).

Under constant mutation effects, the estimates are unbiased with relatively small sampling errors. $\hat{U} = 1.58$ (0.42), $\hat{h} = 0.361$ (0.023), $\hat{s} = 0.030$ (0.008) and $\hat{V}_m = 1.75\text{E-}4$ (0.38E-4). The reported values are the mean and 1 SD of the estimates over the 100 simulations. If only 30 outcrossed parents are sampled as is the case is the present study, $\hat{U} = 2.19$ (3.49), $\hat{h} = 0.363$ (0.057), $\hat{s} = 0.033$ (0.019) and $\hat{V}_m = 1.75\text{E-}4$ (0.96E-4).

Mutation effects $h$ and $s$ are unlikely constant. For example, $s$ may vary anywhere from 0.0 (neutral mutation) to 1.0 (lethal mutation). The rate of occurrence for mutations with different effects may also vary. Under an exponentially distributed rate for mutations of variable effects of $s[\mu(s) = 1/\bar{s}\exp(-s/\bar{s})]$, and letting $h(s) = \frac{1}{2}\exp(-13s)$, which is in rough accordance with the few available data (DENG and LYNCH 1996a), $\hat{U}$ and $\hat{\bar{h}}$ are underestimated, and $\hat{\bar{s}}$ and $\hat{V}_m$ are overestimated, and the sampling errors are relatively small: $\hat{U} = 0.766$ (0.103), $\hat{\bar{h}} = 0.221$ (0.010), $\hat{\bar{s}} = 0.072$ (0.010) and $\hat{V}_m = 1.88\text{E-}4$ (0.31E-4). Please note here, under the variable mutation effects, $V_m = U(\bar{h}\bar{s})^2$ does not hold, due to the nonlinear relationship of $V_m$ with $h$ and $s(V_m = U(hs)^2)$ under constant mutation effects. If the probability density function of $s$ is $f(s)$, and $h$ is a function of $s(h(s))$, $V_m = \int U(sh(s))^2 f(s)\,ds$. With exponentially distributed $s$ effects and the above function for $h(s)$, it can be shown that

$$V_m = \frac{U_s^{-2}}{2(26\bar{s} + 1)^3}.$$

Therefore, the parameter value $V_m$ is 1.20E-4 for the simulated $U$ and $f(s)$ and $h(s)$. No lethal mutations are simulated here.

The effects of synergistic epistatic mutation effects on estimation are simulated by employing the fitness function

$$W(n) = \exp(-\alpha n - \frac{\beta n^2}{2}),$$

where $n = n_1 + (n_2/h)$, and $n_1$ and $n_2$ are the number of loci heterozygous and homozygous for mutations, respectively. Mutation effects are constant and $\alpha = 0.0108$, $\beta = 1.82\text{E-}5$, in which case, the overall synergistic mutation effects are ~10% of the multiplicative mutation effects (DENG and LYNCH 1996a), $\hat{U}$ and $\hat{\bar{h}}$ are underestimated, and $\hat{\bar{s}}$ and $\hat{V}_m$ are overestimated, and the sampling errors are relatively small. $\hat{U} = 1.37$ (0.27), $\hat{\bar{h}} = 0.35$ (0.02), $\hat{\bar{s}} = 0.041$ (0.009) and $\hat{V}_m = 2.73\text{E-}4$ (0.61E-4).

## APPENDIX B

In DENG and LYNCH's (1996a) technique, the fitness function is assumed to be multiplicative. If at one locus, the mutant allele frequency at mutation-selection balance is not affected by the absence of sex, it will not be affected by cyclical parthenogenesis. Therefore, the pattern and magnitude of inbreeding depression should be the same regardless of whether the species reproduces purely sexually or cyclically parthenogenetically, as should the mutation parameters.

At one locus, let $A$ be the wild-type allele and $a$ be the mutant allele, and the fitness of $AA$ be 1, $Aa$ be 1-$hs$, and $aa$ be 1-$s$. Let $u$ be the mutation rate from allele $A$ to allele $a$, and ignore the back-mutation from $a$ to $A$. Under the assumptions that $u$ is small, mutant $aa$ homozygotes are very rare and the frequency of $a(q)$ is mainly determined by selection against heterozygotes. The frequency of $a$ at mutation-selection balance is then approximately $u/hs$ in a randomly mating and purely sexual species (CROW and KIMURA 1970). With the same assumptions (*i.e.*, $aa$ is very rare, and $u$ is small) in asexual species, let the frequency of $AA$ be $P$, then after one asexual generation of selection and mutation, the frequency of $AA$ will be

$$P' \cong \frac{P^*(1 - 2u)}{P^*(1 - 2u) + 2^*p^*u + (1 - P)^*(1 - h^*s)}$$

$$\cong \frac{P^*(1 - 2u)}{1 - h^*s + h^*s^*p},$$

where $p = 1 - q$. At mutation-selection balance, $P' = P$, we have

$$\hat{P} = 1 - \frac{2u}{h^*s},$$

where the circumflex ( ˆ ) indicates the equilibrium value. Thus at mutation-selection balance, the frequency of allele $a$ is $u/h^*s$, which is the same as that in a purely sexual species. Therefore, at a locus, as long as a mutation has fitness effects in the heterozygous state, or equivalently, the dominance coefficient is not zero, sexual reproduction does not alter the mutant allele frequency at mutation-selection balance. Almost all mutations do have heterozygous fitness effects; the smaller the $s$, the bigger the $h$ (KACSER and BURNS 1981), and even for lethal mutations, $h$ is not 0 (CROW and KIMURA 1970, MUKAI *et al.* 1972; CROW and SIMMONS 1983; KONDRASHOV 1988).

The following two results are consistent with the above conclusion. (1) The magnitude of inbreeding depression for the two study populations of cyclical parthenogens is similar to that of other purely sexual species (see text). (2) KIMURA and MARUYAMA (1966) also showed that mutation load is about the same for sexual and asexual populations in the absence of epistasis for mutations. Their results (derived by a different approach) deal with the mean fitness only, while the present proof concerns both the mean and genetic variance by considering equilibrium gene frequency on single loci under different reproduction modes.