

Inferring Deleterious-Mutation Parameters in Natural *Daphnia* Populations

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ABSTRACT

Deng and Lynch (1, 2) proposed to characterize deleterious genomic mutations from changes in the mean and genetic variance of fitness traits upon selfing in outcrossing populations. Such observations can be readily acquired in cyclical parthenogens. Selfing and life-table experiments were performed for two such *Daphnia* populations. A significant inbreeding depression and an increase of genetic variance for all traits analyzed were observed. Deng and Lynch's (2) procedures were employed to estimate the genomic mutation rate (\bar{U}), mean dominance coefficient (\bar{h}), mean selection coefficient (\bar{s}), and scaled genomic mutational variance ($\frac{V_m}{V_e}$). On average, \hat{U} , \hat{h} , \hat{s} and $\frac{\hat{V}_m}{V_e}$ ($\hat{}$ indicates an estimate) are 0.84, 0.30, 0.14 and 4.6E-4 respectively. For the true values, the \hat{U} and \hat{h} are lower bounds, and \hat{s} and $\frac{\hat{V}_m}{V_e}$ upper bounds.

INTRODUCTION

Estimates of the genomic mutation rate to mildly deleterious alleles (\bar{U}) are crucial to testing theories for the evolution of sex (3-6), mate choice (5, 7, 8), outbreeding mechanisms (7), diploidy (9), and the accelerated extinction rate of small populations (10-13). However, few estimates are available (5, 14, 15). The traditional mutation-accumulation experiment (16, 17) takes extensive time and labor. Estimation in highly selfing plants by making use of inbreeding depression data (18) depends on an unknown mean dominance coefficient (\bar{h}) of deleterious mutations. \bar{h} cannot be estimated without bias (1, 17, 19-22). Furthermore, estimation of \bar{U} by inbreeding depression data is very sensitive to the \bar{h} value estimated or assumed (23). 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 ($\frac{V_m}{V_e}$), is also important (24, 12, 25).

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Recently, Deng and Lynch (1, 2) developed an approach, which is shown (23) to be generally more efficient than other currently existing ones (16-18, 27). This approach uses the data (changes of the mean and the total genetic variance for fitness traits upon selfing/outbreeding) in outcrossing/highly selfing populations, to estimate not only U , but also \bar{h} , \bar{s} , and $\frac{V_m}{V_e}$. An unbiased estimate of the total genetic variance requires that clones of the genotypes be available (28) and distributed randomly across the experimental environment. These ensure that there will not be any common environmental effects for clonal members and that the environmental variation will be clearly separated from the total genetic variance in analyses.

In Deng and Lynch's (1) original procedure in outcrossing populations, multiple selfed progeny must be obtained from each selfing parent. The genetic variation employed in estimation in the selfed offspring generation is that among the mean of selfed progeny of selfing families. However, multiple selfed progeny are not always easy to obtain for all the selfing families. Deng and Lynch (2) extended the approach so that only one selfed progeny is required from each selfing parent. The extension is found to be generally more efficient (26).

Cyclical parthenogens are ideal for the application of Deng and Lynch's (1, 2) technique. In cyclical parthenogens, genotypes can be cloned by asexual reproduction, and selfed progeny can be constructed by mating clonal members (which is genetically equivalent to selfing). Thus, outcrossed and selfed genotypes can be assayed simultaneously in one controlled environment, each having multiple replicates. Change of the genetic parameters across generations will then 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.

Selfing and life-table experiments were performed on populations of two cyclically parthenogenetic species of the freshwater cladoceran, *Daphnia arenata* and *Daphnia pulex*. The mean and genetic variance in the outcrossed parental and selfed offspring generations were estimated and used to infer deleterious genomic mutation parameters by Deng and Lynch's (2) extended procedure.

MATERIALS AND METHODS

Study organism and populations: In nature, mating in cyclically parthenogenetic *Daphnia* populations is usually random (29-31) and the population size is effectively infinite (29, 30). In the laboratory, parthenogenetic reproduction can be maintained indefinitely as long as environmental conditions remain favorable. During parthenogenetic reproduction, genotypes are faithfully replicated (31), which makes it possible to estimate the total genetic variance without bias (32, 33). Sexual reproduction can be experimentally induced, and the resultant resting eggs can be hatched (33-39). Generation time is about 2 weeks at 20°C (40).

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 (33, 39).

Experimental procedures

Detailed experimental procedures of sampling populations, cloning and selfing genotypes, determining species identity, and hatching selfed resting eggs have been documented previously (2, 33, 39). Briefly, females sampled from the field were isolated into individual beakers containing about 200 mls of filtered and aged water from the populations' source habitats, and fed with the green alga *Scenedesmus*. The species identity was determined by morphological (41, 42) and biochemical (43-45) criteria. Over a period of two 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 asexually, 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 (33, 36, 39). The hatched selfed individuals were then expanded clonally.

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 (32). All clonal replicates were randomly distributed in the experimental setting. Each clonal replicate was maintained in 100 ml water (aged for at least one 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°C (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°C (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, new-born 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 data were subject to one-way ANOVA to estimate the genetic variance. The estimates of the standard errors for the genetic variance were obtained by the Taylor approximation (32, 37).

Fecundity is believed to be under directional natural selection to increase. Thus, the genotypic means (\bar{W}) and genetic variances (V_g) for fecundity in the selfed offspring generation (s) and the outcrossed

parental generation (p) were used to infer U , \bar{h} , \bar{s} , and $\frac{V_m}{V_e}$ by Equations A1 (4-5) in Deng and Lynch

(2). Due to the complex nonlinear functional relationship of the mutation parameters with the observed quantities in both generations, 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 (1, 2). Simulation results (2, 26) suggest that 200 clones would result in much better estimates with reasonably small sampling errors.

RESULTS

For the Dorena *D. pulicaria* population, selfing resulted in a high magnitude of inbreeding depression for all traits analyzed (Table 1). The results for the change of genetic variance upon selfing 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.

Table 1: Summary of the mean (\bar{W}) and genetic variance (V_g) for fitness traits for the selfed progeny generation (s) and outcrossed parental generation (p)

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

The numbers within parentheses indicate one standard error

Estimates for U , \bar{h} , \bar{s} and $\frac{V_m}{V_e}$ are quite consistent within each population but differ somewhat between the two populations, especially for \bar{s} (Table 2). Averaging over the first three clutches, \hat{U} , \hat{h} , \hat{s} and $\frac{\hat{V}_m}{\hat{V}_e}$ were 0.99, 0.36, 0.21 and 8.9E-4 respectively for the D. pulicaria population, and 0.69, 0.23, 0.07, 3.4E-5 respectively in the D. arenata population. Averaging over the two populations, \hat{U} , \hat{h} , \hat{s} and $\frac{\hat{V}_m}{\hat{V}_e}$ were 0.84, 0.30, 0.14, and 4.6E-4 respectively.

Table 2 : Estimates for U , \bar{h} , \bar{s} and $\frac{V_m}{V_e}$

Fitness trait	\hat{U}	\hat{h}	\hat{s}	$\frac{\hat{V}_m}{\hat{V}_e}$
Dorena D. pulicaria				
1st clutch size	0.79	0.35	0.20	1.2E-3
2 nd 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
2 nd clutch size	0.38	0.20	0.07	1.4E-5
Average	0.69	0.23	0.07	3.4E-5

Genetic correlations between fecundities of different clutches ($R_G(C_i, C_j)$) = total genetic correlation between the *i*th and *j*th clutch sizes) are high. $R_G(C_1, C_2) = 0.67$, $R_G(C_2, C_3) = 0.91$, $R_G(C_1, C_3) = 0.78$ for the Dorena D. pulicaria population, and $R_G(C_1, C_2) = 0.53$ for the Amazon D. arenata population. 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.

DISCUSSION

The study adds inbreeding data from cyclical parthenogens to the existent abundant inbreeding literature in purely sexual organisms (7, 28). Most importantly, the experiments here provide a demonstration,

though the data are still preliminary, that the estimation techniques developed by Deng and Lynch (1, 2) are promising in characterizing deleterious genomic mutations in natural populations.

Due to the potential variable mutation effects and epistatic effects (1, 2), the genomic mutation parameters estimated here are lower bounds in the case of \hat{U} , \hat{h} , and upper bounds in the case of \hat{s} and $\frac{\hat{V}_m}{V_e}$. However, the estimates are not biased by the peculiar life cycle of cyclical parthenogens (2).

All of the assumptions used by our estimation approach are essentially the same as those used by Charlesworth et al. (18); some are the same as those employed in the traditional mutation-accumulation approach (16, 17, 46). 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 gametic 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 experiments here were performed by the author with some assistance in a total of about 12 months. Larger scale experiments such as those employing ~200 clones should be carried out to achieve estimates with relatively small sampling errors.

It should be noted that selfing is not essential with our approach (26), though cloning of genotypes still is in order to separate environmental variance from genetic variance. We (26) have worked out the exact formulation for experimental settings of any degree of inbreeding, so that as long as genotypes can be cloned, our approach can be applied. In addition, we (Deng et al., in prep.) are developing appropriate experimental designs and statistical analyses that may eliminate the requirement of cloning genotypes so that characterizing deleterious mutations can be accomplished in almost any outcrossing populations. Furthermore, it seems possible to obtain estimates of deleterious genomic mutations that may not be biased by the variable mutation effects (Deng et al., unpublished).

Our estimation (1, 2) approaches were investigated extensively by computer simulations for robustness, statistical properties and different designs (1, 2, 26), while many of these issues imperatively await studies for the other estimation methods (16-18, 27, 47). The most important assumption underlying the estimation applying to natural populations (1,2,18,27) is that populations are at mutation-selection balance. Accordingly, all the standing genetic variation is due to directional selection on deleterious genomic mutations and heterosis/inbreeding depression is all due to dominance. Even in large populations, despite tremendous efforts (23, 48-52), the extent to which this assumption is valid is generally unknown. In order to correctly characterize deleterious genomic mutations from natural populations (1,2,18,27), this assumption needs to be examined closely—a task that is being actively pursued (53).

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