Effects of a change in the level of inbreeding on the genetic load

S. C. H. Barrett* & D. Charlesworth†

* Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada
† Department of Ecology and Evolution, University of Chicago, 5630 S. Ingleside Avenue, Chicago, Illinois 60637, USA

"THE effects of inbreeding may not be as noticeable in the first generation as the invigoration immediately apparent after crossing"[1]. This statement, published in 1919, has received little attention, and has apparently never been tested empirically, although the reduction of the genetic load of populations by inbreeding is well known in theoretical terms[2–5]. Because inbreeding increases homozygosity, and hence the effectiveness of selection against recessive or partially recessive detrimental alleles, changes in levels of inbreeding can lead to a reduction in the frequencies of such mutant alleles. This results in equilibration at higher population mean fitness[6] and is referred to as 'purging' populations of their genetic load. Severe inbreeding can also reduce genetic load due to overdominant alleles, provided selection coefficients are not symmetrical at all loci, because alleles giving lower fitness will be reduced in frequency at equilibrium[7,8]. With either fitness model, however, reduction in genetic load takes time, and the initial effect of an increase in inbreeding is reduced fitness due to homozygosity. There are few data relating to the extent to which fitness is reduced during inbreeding in a set of lines and to how long the reduction lasts before increasing again to the initial level, or higher. Inbreeding experiments involving sib mating in mice and *Drosophila subobscura*[10], and successive bottlenecks in house flies[11] have yielded some evidence consistent with the purging hypothesis. Here, we report results of an experiment demonstrating a prolonged time-course of recovery of mean fitness under self-fertilization of a naturally outcrossing plant, and also compare our results with expectations derived by computer calculations. Our results show that the genetic load present in an outcrossing population can be explained only with a high mutation rate to partially recessive deleterious alleles, and that inbreeding purges the population of mutant alleles.

The experimental plant was the self-compatible annual water hyacinth, *Eichhornia paniculata* (Pontederiaceae). The species inhabits ephemeral pools and ditches in northeastern Brazil and the Caribbean, and exhibits a wide range of natural outcrossing rates[12]. The experiments reported here used material from two populations with contrasting breeding systems. Population B11 from Brazil had an estimated outcrossing rate of 0.94, whereas

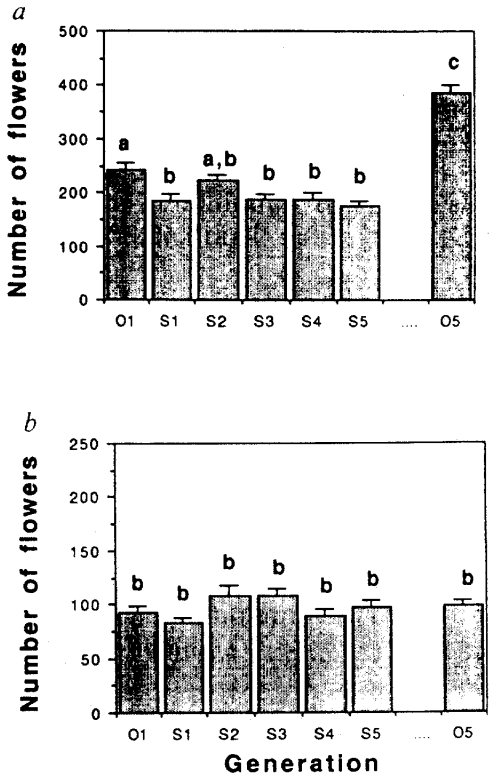

FIG. 1 Mean flower numbers of plants in various inbred and intercrossed generations. *a*, Naturally outbred population B11, NE Brazil. *b*, Naturally inbred population J13, Jamaica.

METHODS. Seed from open-pollinated families were randomly sampled from the two populations, B11 and J13. Lines were established in the glasshouse from each of 30 families from B11, and from each of 10 families from J13. One plant from each line was selfed and randomly outcrossed to other plants from the same population, to give the S_1 and O_1 generations, respectively. A small amount of S_1 seed was sown and a single randomly chosen plant was self-pollinated to give the S_2 generation. The remaining S_1 and O_1 seeds were stored dry at room temperature. The selfing was repeated for four successive generations with seeds from the S generations being stored. Plants from the lines were then selfed and outcrossed (within populations) to give the S_5 and O_5 generations. Stored seeds (maximum age 3 years) from all generations were then sown and the multi-generation plants grown in the glasshouse under uniform conditions. Fitness components were compared among generations in a randomized block design with all lines represented. Sample sizes were $n = 1007$ for population B11 (average 4.8 plants per line per generation), and $n = 378$ for J13 (average 5.4 plants per line per generation). Means and standard errors are shown, and the letters above the bars indicate statistically significant differences (5% level).

TABLE 1 Differences in mean fitness between different generations in our inbreeding experiments and models, and in experiments with maize[17] and mice[9].

Source of data	$Inbred_1$	$Inbred_5$	O_5
E. paniculata (flower number)			
Population B11	0.761	0.732	1.597
Population J13	0.901	1.053	1.062
Maize (yield)			
1909	—	0.447	1.072
1910	—	0.394	1.124
1911	—	0.293	1.393
Mice (litter size)			
F_1	—	0.924	1.021
F_2	—	0.923	1.155
Theoretical			
Mutational model, initially completely outcrossing			
$s=0.2$, $h=0.2$, $U=1.0$	0.482	0.631	1.436
$s=0.2$, $h=0.1$, $U=1.0$	0.147	0.191	1.433
$s=0.2$, $h=0.2$, $U=0.5$	0.746	0.835	1.187
$s=0.9$, $h=0.2$, $U=0.1$	0.930	1.038	1.066
Mutational model, initial outcrossing rate of 0.9			
$s=0.2$, $h=0.2$, $U=1.0$	0.559	0.655	1.278
Overdominance model			
4 loci, $s_1=0.1$, $s_1=0.2$	0.873	0.782	0.994

Outcrossed generations are denoted by O, and inbred generations by "Inbred", and the numbers indicate the generation number when the fitness was estimated (see legend to Fig. 1). The fitness values are all expressed relative to the values for the initial outcrossed population (O_1), but for the maize and mouse data, values from families that were maintained by enforced cross-breeding are used. The numbers of generations of inbreeding for the maize experiment are not given in ref. 17, but there were certainly several generations of inbreeding. For the mouse experiment, the inbreeding coefficient was also non-zero (at least 0.3) when the experiment was stated[9], and the data come from 11 generations of sib-mating followed by intercrossing to form F_1 and F_2 generations. The methods used to generate the theoretical results are given in the legend to Fig. 2.

population J13 from Jamaica was highly selfing, with no within-population heterozygosity observed at 21 allozyme loci[13]. A range of life-history traits was measured on all plants during the 8-month experiment. Only data on mean flower number per plant are presented here, but results for biomass were similar. Flower number exhibits the strongest inbreeding depression in *E. paniculata*. It represents the outcome of many developmental steps and must be influenced by many loci. As the species is annual, this trait is likely to be highly correlated with fitness. The results of five generations of self-fertilization of plants from population B11, followed by intercrossing the inbred lines, are shown in Fig. 1. Inbreeding had no significant effect on flower number per plant from the naturally inbreeding J13 population (Fig. 1; Table 1), showing that the differences observed are unlikely to be due to ageing of the stored seeds.

The expectations for such an experiment were calculated using deterministic computer programs that model either over-dominant selection at several loci[14], or mutation-selection balance at many unlinked loci[5]. There was remarkable similarity between the main features of the experimental results and the theoretical expectations of the mutational model for the inbred generations, when the initial populations before inbreeding begins were subject to a high mutation rate to moderately detrimental, partially recessive alleles (Figs. 1, 2; Table 1). Mean fitness values decreased in the first generation of inbreeding, but subsequently remained at roughly the same level. After five generations of inbreeding, the mean fitness of progeny derived from intercrossing the inbred lines was much higher than that of the inbred progeny, and also considerably higher than the fitness of the original outbred population. With strongly deleterious mutations (selection coefficient, 0.9), rapid recovery of fitness during the five generations of inbreeding was clearly evident (Fig. 2*b*; Table 1), unlike the experimental results. With

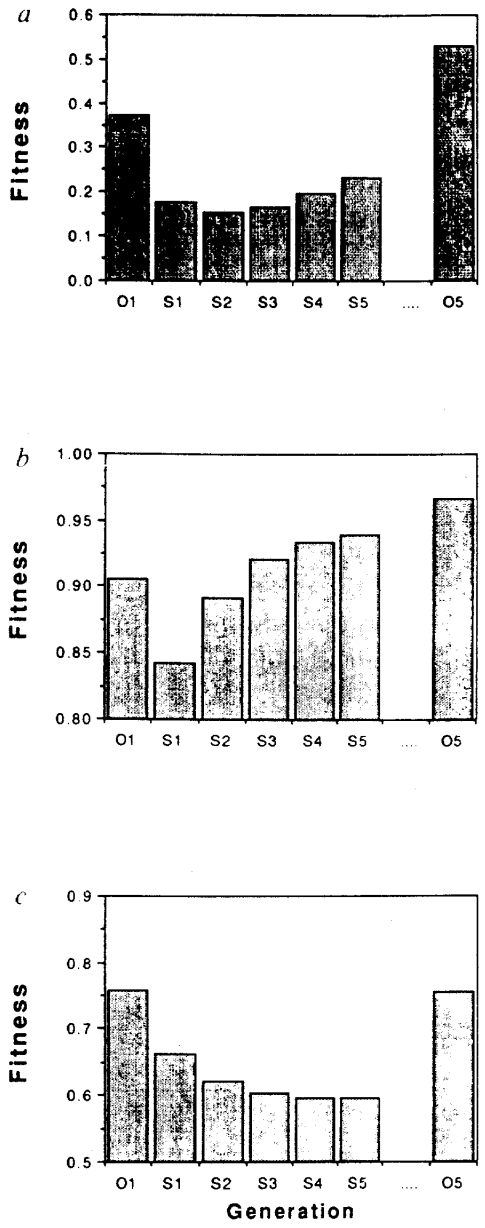

FIG. 2 Theoretical results for different selfed and intercrossed generations, assuming unlinked loci. *a*, Mutational model: selection coefficient $s=0.2$; dominance coefficient $h=0.2$; mutation rate per diploid genome $U=1.0$. *b*, Mutational model: $s=0.9$; $h=0.2$; $U=0.1$. *c*, Overdominance model: selection coefficients $s_1=0.1$ and $s_2=0.2$ at four loci.

METHODS. The computer calculations for the mutational model were obtained using the infinite population size model of Kondrashov[24] programmed as described[5]. In this model, the fitness of a homozygote for a mutant allele at one locus was denoted by $1-s$, where s is the selection coefficient, and the fitness of heterozygotes by $1-hs$, where h is the dominance coefficient. For the overdominance model, a five-locus deterministic program was used, with symmetric selection coefficients s_1 and s_2 (with the biologically implausible assumption of symmetrical overdominance, the mean fitness declines with inbreeding, even though variation is maintained with both alleles at frequencies of 0.5, and the fitness on intercrossing inbred lines will not exceed the original outbred value). To combine the fitness effects of different loci, multiplicative fitnesses were assumed in both models. For each model populations were run until equilibrium was reached under almost complete outcrossing (outcrossing rate 0.99), and then the selfing rate was altered to a high value (outcrossing rate 0.01). The time-course of changes in the population was then followed. Each generation, the fitnesses of inbred and outbred progeny produced in the population were calculated.

a partially inbred initial population, the results were similar to those observed (Table 1).

With the asymmetrical overdominance model, populations at equilibrium under high selfing do not maintain variation, but will fix the allele least disadvantageous in homozygotes though an increase will occur if the set of lines has not reached equilibrium, so that some still remain with the lower fitness allele. One would thus expect no increase in fitness on intercrossing the inbred lines after the population reaches equilibrium. During inbreeding, the fitness values behaved similarly to those in the mutational model, with a slight decrease, rather than increase, in fitness (Fig. 2*c*; Table 1). But the intercross mean fitness, although higher than the inbred fitness, was never higher than the fitness of the initial population, unlike the mutational load model and the observed data (Table 1).

These results support the partial dominance (mutational) hypothesis[2,7] for genetic load, and suggest that the mutation rate per genome in *E. paniculata* must be very high (of the order of one per generation). This is consistent with most other available data[15,16]. The failure of fitness to recover during inbreeding, and intercrossed fitness levels higher than those of the original outbred strains, are also seen in maize[17] and mice[9] (Table 1). This suggests that purging of partially recessive mutations accounts for an important component of heterosis. When the fitness values under inbreeding are plotted against the inbreeding coefficient, the partial dominance model yields an increase with high F values, due to purging, but the overdominance model predicts a monotonic decline. The partial dominance model can thus also explain the observed patterns in inbreeding experiments with conifers[18].

The occurrence of natural selection during the inbreeding experiment, consistent with purging, was indicated by excess heterozygosity of five (presumably neutral) allozyme marker loci scored in the experimental plants, each generation of inbreeding (data not shown). Under inbreeding, heterozygotes tend to be produced by outcrosses. Surviving adults in the progeny generation therefore tend to be heterozygotes[19,20], and excess heterozygosity compared with the neutral expectation is frequently found in inbreeding plants[21].

These results are also relevant to populations undergoing disturbance by humans. Inbreeding of formerly outbred populations occurs in many zoo populations, as well as domesticated populations and populations subjected to severe reductions in size as a result of human alterations of the environment. Extreme inbreeding has been recommended to purge genetic load and force the adaptation of endangered populations to the inbreeding regime they will experience under human management[22]. This assumes that lowered fitness inevitably caused by increased homozygosity during this process will not be too severe or prolonged. The validity of this assumption depends on the severity and dominance of the mutant alleles in the population being inbred[23,5]. Our results indicate that purging by the most severe inbreeding, self-fertilization, could decrease fitness considerably, with little recovery under inbreeding, and that fitness is restored only when the inbred lines are intercrossed. □

Received 10 June; accepted 21 June 1991.

1. East, E. M. & Jones, D. F. *Inbreeding and Outbreeding. Their Genetic and Sociological Significance* 147 (Lippincott, Philadelphia, 1919)
2. Crow, J. F. *Genetics* **33,** 477–487 (1948).
3. Wright, S. *Evolution and the Genetics of Populations* Vol. 3 (Univ. of Chicago Press, 1977)
4. Lande, R. & Schemske, D. W. *Evolution* **39,** 24–40 (1985)
5. Charlesworth, D., Morgan, M. T. & Charlesworth, B. *Evolution* **44,** 1469–1489 (1990).
6. Crow, J. F. in *Mathematical Models in Population Genetics* (eds Kojima, K.-I.) 128–177 (Springer-Verlag, Berlin, 1970).
7. Charlesworth, D. & Charlesworth, B. *A. Rev. ecol. Syst.* **18,** 237–268 (1987)
8. Ziehe, M. & Roberds, J. H. *Genetics* **121,** 861–868 (1989)
9. Falconer, D. S. *Genet. Res. Camb.* **17,** 215–235 (1972)
10. Hollingsworth, M. J. & Maynard-Smith, J. *J. Genet.* **53,** 295–314 (1955)
11. Bryant, E. H., Meffert, L. M. & McCommas, S. A. *Amer. Nat.* **136,** 542–549 (1990)
12. Barrett, S. C. H. & Husband, B. C. *Pl. Spec. Biol.* **5,** 41–55 (1990)
13. Glover, D. E. & Barrett, S. C. H. *Heredity* **59,** 7–17 (1987)
14. Charlesworth, D. & Charlesworth, B. *Evolution* **44,** 870–888 (1990)
15. Kondrashov, A. *Nature* **336,** 435–440 (1988)
16. Charlesworth, B., Charlesworth, D. & Morgan, M. T. *Nature* **347,** 380–382 (1990)
17. Shull, G. H. in *Heterosis* (ed. Gowen, J. W.) 14–48 (Iowa University Press, Ames, 1950)
18. Griffin, A. R. & Lindgren, D. *Theor. appl. Genet.* **71,** 334–343 (1985)
19. Ohta, T. & Cockerham, C. C. *Genet. Res. Camb.* **23,** 191–200 (1974).
20. Sing, C. F., Brewer, G. J. & Thirtle, B. *Genetics* **75,** 381–404 (1973).
21. Brown, A. H. D. *Theor. appl. Genet.* **15,** 1–42 (1979).
22. Templeton, A. R. & Read, B. *Zoo Biol.* **3,** 177–199 (1984)
23. Wilton, A. N., Joseph, M. G. & Sved, J. *Genet. Res. Camb.* **54,** 129–140 (1989).
24. Kondrashov, A. S. *Genetics* **111,** 635–653 (1985).

ACKNOWLEDGEMENTS. This research was funded in part by an operating grant from the Natural Sciences and Engineering Research Council of Canada (to S.C.H.B.) and in part by an NSF grant to D.C. We thank W. Cole for technical assistance and advice, and B. Charlesworth for his comments on the manuscript.