

# Negative Effects of Inbreeding of Artificially Bottlenecked *Drosophila melanogaster* Populations

Baek-Jun Kim\* 

National Institute of Ecology, Seocheon, Korea

## ABSTRACT

Detrimental effects of inbreeding have been studied by many researchers for a long time. However, only a few studies have shown the occurrence of inbreeding depression due to evolutionary changes as a purging process. In this study, two different populations (inbreeding and outbreeding) of *Drosophila melanogaster* were compared to assess inbreeding effects on artificial population bottlenecks. For inbreeding conditions, a couple of *D. melanogaster* (one virgin and one male) were selected from an inbred population and cultured in a vial. For outbreeding conditions, a couple of *D. melanogaster* were selected from different populations and cultured in a vial. There were significant differences in body lengths of adults, but not in other parameters such as the total number of adults, the rate of survival, and the rate of wing mutants. The mean body length of adults of outbreeding populations was longer than that of inbreeding populations in the first generation (G1;  $P = 0.004$ ), but not in the second generation (G2;  $P = 0.066$ ). Although the other three parameters (total number of adults, rate of survival, and rate of wing mutants) showed differences in their mean values between inbreeding and outbreeding populations, these differences were not statistically significant. This might be due to genetic purging. This study demonstrated one additional experimental case related to inbreeding depression in artificial bottlenecked populations. Further studies are necessary to confirm the clear interaction between inbreeding depression and genetic purging using more generations and replicates (or samples) of *D. melanogaster*.

**Keywords:** Body length, Bottleneck, Fitness, Fruit fly, Inbreeding, Outbreeding

## Introduction

Detrimental effects of inbreeding have been studied for many centuries. They have been subjects of extensive discussion from a number of perspectives (Charlesworth & Charlesworth, 1987; Darwin, 1876; 1885; Lerner, 1954). The loss of fitness that accompanies the increase in homozygosity due to inbreeding is referred to as inbreeding depression. Most fitness effects are thought to be due to increased homozygosity for recessive deleterious alleles (Charlesworth & Charlesworth, 1987). The level of inbreeding depression expressed for a given population mainly depends on the rate of inbreeding (Bijlsma *et al.*, 1999). Although the phenomenon of inbreeding depression

has been observed, only a few studies have shown the occurrence of inbreeding depression in natural populations (Bijlsma *et al.*, 1999). Inbreeding problems for small natural populations are much less than expected because populations can reduce or eliminate inbreeding depression by evolutionary changes called purging (Bijlsma *et al.*, 1999). In addition, several studies have suggested that inbreeding depression strongly depends on environmental conditions and that the inbreeding load becomes more visible under more stressful conditions (Bijlsma *et al.*, 1999). Thus, the consequences of inbreeding will depend not only on the previous history of inbreeding and selection, but also on the prevailing environmental conditions (Bijlsma *et al.*, 1999).


When a population passes through a generation or several generations with a small population size termed as a population bottleneck, genetic drift can reduce the overall heterozygosity, the number of alleles for a trait (by fixation of one of the alleles and loss of the others), and the amount of additive genetic variation in the population (Ja-

**Received** March 23, 2021; **Revised** April 2, 2021;

**Accepted** April 2, 2021

\*Corresponding author: Baek-Jun Kimi

e-mail [naturalist71@nie.re.kr](mailto:naturalist71@nie.re.kr)

 <https://orcid.org/0000-0002-8207-1931>

mes, 1970; Nei *et al.*, 1975). This loss of genetic diversity is thought to have negative consequences on population fitness by fixing detrimental variations and reducing genetic variations (Miller & Hedrick, 2001). However, bottleneck is also known as a potential mechanism of reorganizing genetic diversity (Carson & Templeton, 1984). It is generally believed that bottlenecks can result in the decrease of population fitness and that the possibility of increase of additive genetic variation is quite limited (López-fanjul *et al.*, 1999).

In general, fitness is reduced for bottlenecked populations due to random changes in allelic frequencies. However, it has been suggested that detrimental alleles, primarily responsible for lowered fitness caused by inbreeding or bottlenecks, might be effectively purged by a combination of genetic drift, inbreeding, and selection (Miller & Hedrick, 2001). For example, Templeton and Read (1983; 1984) have found that the initial inbreeding depression for survival in a captive of Speke's gazelle (*Gazella spekei*) is reduced in animals whose parents are inbred. Bryant *et al.* (1990) have shown that early inbreeding depression within serially bottlenecked isofemale lines of the housefly (*Musca domestica*) is largely ameliorated after later bottlenecks. Saccheri *et al.* (1996) have mentioned that a low hatching rate in a butterfly (*Bicyclus anynana*) caused by inbreeding is generally eliminated in later generations. However, this increase in fitness after inbreeding has not always been evident. For instance, Ballou (1997) has investigated 25 captive species and found little effect of past inbreeding on fitness. In contrast, Byers and Waller (1999) have found little evidence of purging in 52 reviewed studies.

Inbreeding may affect fitness negatively in numerous ways (Dahlgaard & Loeschke, 1997). It has been commonly observed in domestic animals, plants, and laboratory experiments (Bijlsma *et al.*, 2000). Inbreeding can influence most fitness components, causing reduced viability, lower fecundity, increased sterility, decreased mating success, slower development, increased susceptibility to environmental stress, and consequently leading to a significant decrease in individual fitness (Bijlsma *et al.*, 2000). In contrast, the process of purging can reduce genetic load and weaken inbreeding depression, resulting in a higher mean fitness of the population (Bijlsma *et al.*, 2000). Inbreeding populations could potentially overcome and eliminate negative effects of inbreeding by this process. However, it is generally expected that purging cannot prevent inbreeding populations from becoming homozygous for deleterious alleles. Thus, it cannot prevent reduced mean fitness of such populations (Barret & Charlesworth, 1991). In addition, although purging is reasonably effective, it has been recently shown that its eff-

ectiveness is environment-dependent and restricted to the environment in which purging occurs (Bijlsma *et al.*, 1999). Purging may possibly be effective in mitigating fitness effects of inbreeding in a short-term, but not in a long-term. These studies indicate that environmental and demographic effects should be considered when evaluating genetic effects of inbreeding.

In this study, different effects of inbreeding and outbreeding of bottlenecked populations of *Drosophila melanogaster* were investigated to confirm results of previous researches. To understand differences between the two populations, the following four parameters were evaluated: body length of adults, total number of adults, rate of survival at the end, and the number of wing mutants.

## Materials and Methods

### Fruit flies

For this experiment, flies from a wild-type population were used. Flies were cultured under standard conditions (temperature, 25–30 °C; relative humidity, 40–60%) using standard potato media containing 3–4 grains of dead yeast and water in each vial. Similar conditions were maintained for all generations. However, water supply was restricted as a limiting environmental factor after flies laid eggs.

### Inbreeding-to-outbreeding experimental procedures

Virgin flies were obtained from a vial containing wild-type flies. For this preliminary work, females were selected within eight hours after hatching to prevent mating. A virgin female and a male were then cultured together as a set of parents (P) in the same vial. Males were selected from non-sib populations and full-sib populations for outbreeding and inbreeding, respectively. A total of 12 vials (with 12 pairs of flies) were used, six for inbreeding populations and six for outbreeding populations in G1. However, a total of 10 vials (with 10 pairs of flies) were used, five for inbreeding populations and five for outbreeding populations in G2. Therefore, there were six or five replicates for G1 and G2 populations, respectively. For inbreeding, a virgin female and a male from the same vial were transferred into a new vial. For outbreeding, the male was obtained from a different vial, although the virgin female was from the same vial. Body length of adults, total number of adults, rate of survival at the end, and rate of wing mutants were recorded for each generation. The body length of adults was measured with a Vernier caliper. The offspring produced by sets of parents (P) were chosen and cultured in each condition. Equal care was provided to all cultures for each generation (G1 and G2). Schematic procedures are shown in Fig. 1.

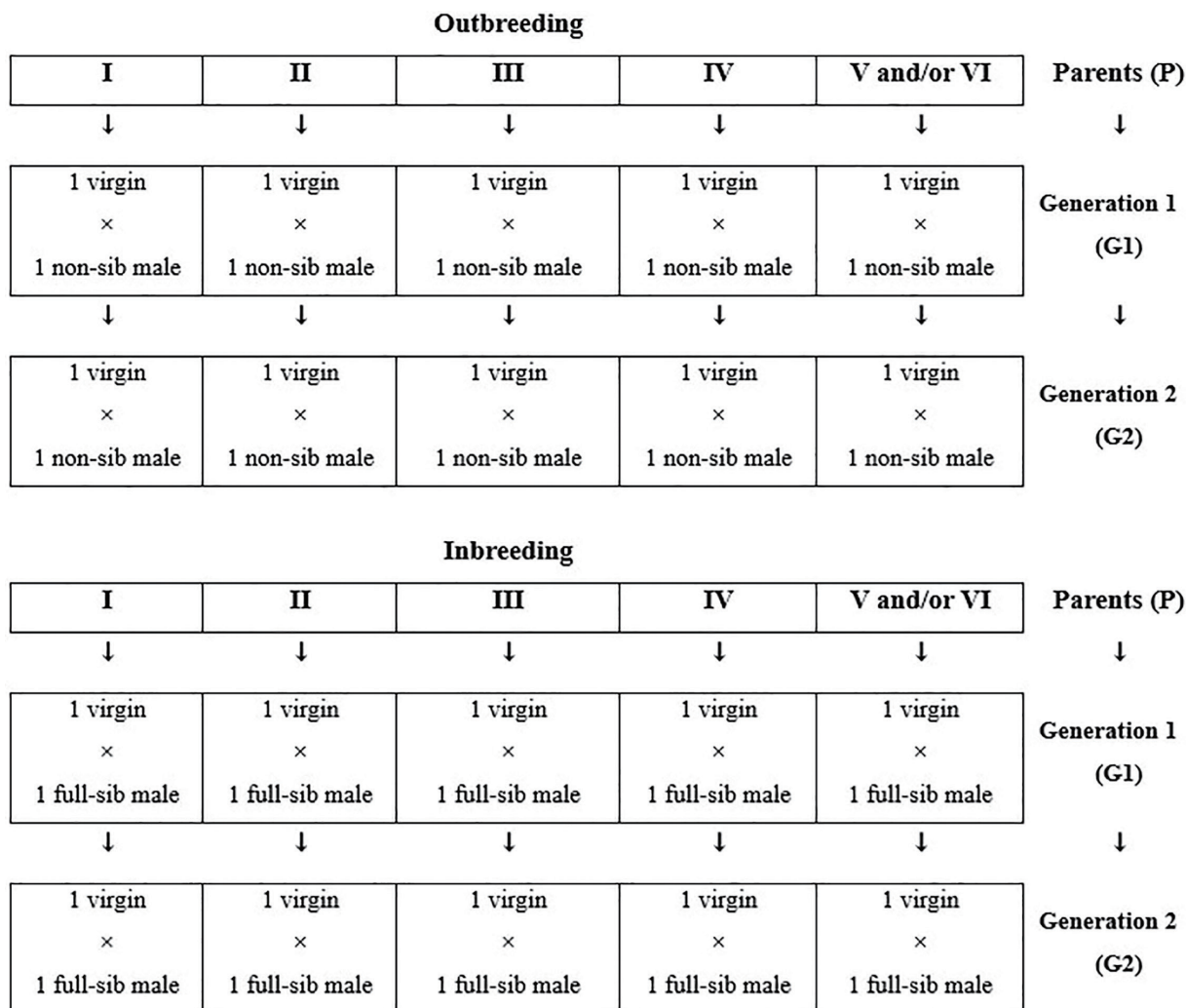


Fig. 1. The schematic procedure of outbreeding and inbreeding in G1 and G2.

### Statistical analyses

For all four parameters, one-way analysis of variance (ANOVA) was performed to compare each population. Tukey's HSD (honest significant difference) test was not performed because there were only two cases for each parameter. Homogeneity and normality were not tested because the sample size was insufficient. However, ANOVA was robust against violation of assumptions of normality and homogeneity of variance in the case of an equal sample size. All data were analyzed using SPSS software version 10.0 (SPSS Inc., U.S.A.).

### Results

There was no significant difference in any parameters except for the mean body length of adults between outbred

and inbred populations of *D. melanogaster* (Fig. 2). The outbreeding populations showed significantly longer body length of adults than the inbreeding populations in G1 (Fig. 2A; ANOVA,  $P = 0.004$ ). Although there was a substantial difference in the mean body length, no significant difference in G2 was found between the two populations (Fig. 2A; ANOVA,  $P = 0.066$ ). There was no significant difference in the three other parameters, such as the total number of adults (Fig. 2B; ANOVA,  $P = 0.631$  and  $0.779$  for G1 and G2, respectively), the rate of survival (Fig. 2C; ANOVA,  $P = 0.444$  and  $0.347$  for G1 and G2, respectively), and the rate of wing mutants (Fig. 2D; ANOVA,  $P = 0.757$  and  $0.480$  for G1 and G2, respectively) between outbreeding and inbreeding populations. There were little differences in mean values of these three parameters between the two populations.

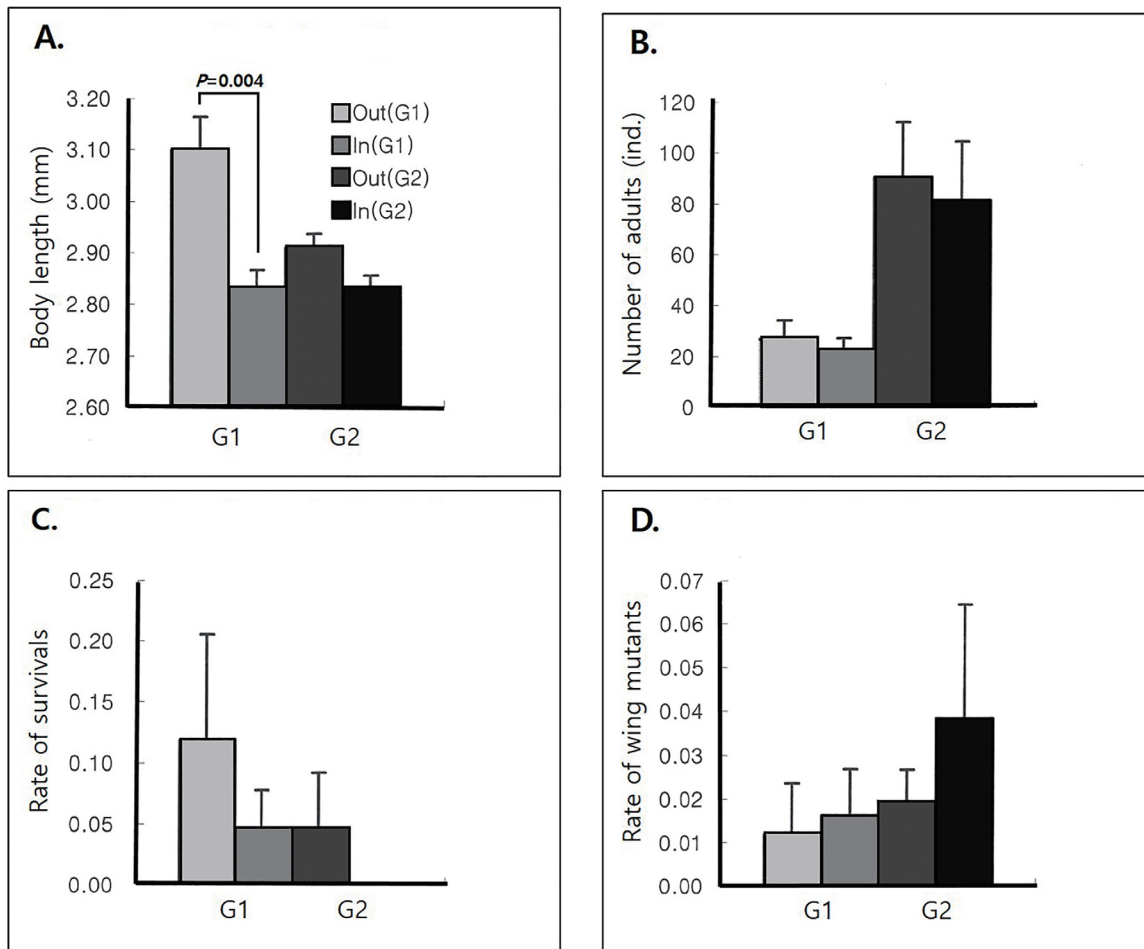


Fig. 2. Differences of each parameter between outbreeding and inbreeding populations in G1 and G2. A. Body length; B. The total number of adults; C. The rate of survival at the end; D. The rate of wing mutants.

### Discussion

Most fitness effects are thought to be due to increased homozygosity for recessive deleterious alleles (Charlesworth & Charlesworth, 1987). Based on studies of *Drosophila* using chromosome balancer techniques (Sved & Ayala, 1970), approximately half cases of inbreeding depression are due to rare recessive lethal or sub-lethal mutations while the rest can be attributed to a large number of mildly deleterious mutations (Charlesworth & Charlesworth, 1987). Other researchers have suggested that inbreeding depression, to some extent, may also result from synergistic or epistatic interactions (Charlesworth, 1998). In this study, inbreeding depression due to recessive mutations, not due to synergistic or epistatic interactions, was focused on.

In this study, there was no significant difference in all parameters except for the body length of adults between

outbreeding and inbreeding populations. Adults of the outbreeding population were longer than those of the inbreeding population in G1. Bijlsma *et al.* (2000) has mentioned that inbreeding can affect most fitness components and lead to reduced viability, lower fecundity, increased sterility, decreased mating success, slower development, and increased susceptibility to environmental stress, consequently resulting in a significant decrease in individual fitness. In this study, a significant difference was only seen in body lengths of adults. There were slight differences in other three parameters. However, such differences were not statistically significant. Keller and Waller (2002) have suggested that if populations remain small and isolated for many generations, they face two genetic threats. First, as alleles are randomly fixed or lost from the population by genetic drift, levels of quantitative genetic variation necessary for adaptive evolution will erode (Lande, 1995). Second, deleterious mutations tend to

accumulate because selection is less effective in small populations (Lynch *et al.*, 1995). This could eventually lead to a mutational meltdown for populations with an effective size ( $N_e$ ) < 100. Both processes tend to be gradual. Thus, they do not threaten populations in the short-term. In this study, inbreeding effects were assessed for only two generations. This might not be sufficient to see effects of inbreeding depression. For extensive data analysis, it is necessary to use more replicates or samples. Five or six replicates were used for the statistical analysis of inbreeding effects in the present study. Other researchers have observed more generations using more replicates or samples to evaluate differences between outbreeding and inbreeding populations. For example, Bijlsma *et al.* (1999) have used nine generations of *Drosophila* to see inbreeding effects. Furthermore, they have observed the maintenance of these effects for approximately 50 generations using 50 replicates for each condition.

It has been suggested that inbreeding problems for small populations are far less than expected because they can reduce or eliminate inbreeding depression by evolutionary changes, termed purging at the contributing loci (Ballou, 1997; Battett & Charlesworth, 1991; Hedrick, 1994; Templeton & Read, 1984). However, several studies have suggested that inbreeding depression strongly depends on environmental conditions and that the inbreeding load becomes more visible under more stressful conditions (Bijlsma *et al.*, 1997; Dahlgaard *et al.*, 1995; Miller, 1994). For example, Miller (1994) has demonstrated that *D. melanogaster* homozygous for the second chromosome shows a significant increase in inbreeding depression under lead stress, whereas the same homozygote in an unleaded environment does not. In this study, water supply was restricted after *D. melanogaster* laid eggs to provide a stressful environmental condition. However, this stressful condition probably had no significant effects on inbreeding except on the mean body length of adults.

This study showed a statistically significant difference in the body length of *D. melanogaster* adults as a proof of negative inbreeding effect. However, the other parameters did not show a significant difference between outbred and inbred populations due to genetic purging. This study demonstrated one additional experimental case related to inbreeding depression in artificial bottleneck populations. For future studies, it might be necessary to use more replicates (or samples) and more generations to see effects of inbreeding depression in bottlenecked populations.

### Conflict of Interest

The author has declared that no competing interests exist.

### Acknowledgments

This work was supported in part by Korea Environment Industry & Technology Institute (KEITI) through The Decision Support System Development Project for Environmental Impact Assessment funded by Korea Ministry of Environment (MOE) (No. 2020002990009) and by the National Institute of Ecology (NIE) funded by Korea Ministry of Environment (MOE) (No. NIE-C-2021-30).

### References

- Ballou, J.D. (1997). Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *Journal of Heredity*, 88, 169-178. doi:10.1093/oxfordjournals.jhered.a023085
- Barrett, S.C., and Charlesworth, D. (1991). Effects of a change in the level of inbreeding on the genetic load. *Nature*, 352, 522-524. doi:10.1038/352522a0
- Bijlsma, R., Bundgaard, J., and Boerema, A.C. (2000). Does inbreeding affect the extinction risk of small population? Predictions from *Drosophila*. *Journal of Evolutionary Biology*, 13, 502-514. doi:10.1046/j.1420-9101.2000.00177.x
- Bijlsma, R.J., Bundgaard, J., Boerema, A.C., and van Putten, W.F. (1997). Genetic and environmental stress, and the persistence of populations. In R. Bijlsma, and V. Loeschcke (Eds.), *Environmental Stress, Adaptation and Evolution (Experientia Supplementum, 83)* (pp. 193-207). Basel: Birkhäuser. doi:10.1007/978-3-0348-8882-0\_11
- Bijlsma, R., Bundgaard, J., and van Putten, W.F. (1999). Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 12, 1125-1137. doi:10.1046/j.1420-9101.1999.00113.x
- Bryant, E.H., Meffert, L.M., and McCommas, S.A. (1990). Fitness rebound in serially bottlenecked populations of the housefly. *The American Naturalist*, 136, 542-549. doi:10.1086/285112
- Byers, D.L., and Waller, D.M. (1999). Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics*, 30, 479-513. doi:10.1146/annurev.ecolsys.30.1.479
- Carson, H.L., and Templeton, A.R. (1984). Genetic revolutions in relation to speciation phenomena: The founding of new populations. *Annual Review of Ecology and Systematics*, 15, 97-132. doi:10.1146/annurev.es.15.110184.000525
- Charlesworth, B. (1998). The effect of synergistic epistasis on the inbreeding load. *Genetics Research*, 71, 85-89.

- doi:10.1017/S0016672398003140
- Charlesworth, D., and Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, 18, 237-268. doi:10.1146/annurev.es.18.110187.001321
- Dahlgaard, J., Krebs, R.A., and Loeschcke, V. (1995). Heat-shock tolerance and inbreeding in *Drosophila buzzatii*. *Heredity*, 74, 157-163. doi:10.1038/hdy.1995.23
- Dahlgaard, J., and Loeschcke, V. (1997). Effects of inbreeding in three life stages of *Drosophila buzzatii* after embryos were exposed to a high temperature stress. *Heredity*, 78, 410-416. doi:10.1038/hdy.1997.64
- Darwin, C. (1876). *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. London: John Murray.
- Darwin, C. (1885). *The Variation of Animals and Plants under Domestication*. 2nd ed. London: John Murray.
- Hedrick, P.W. (1994). Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity*, 73, 363-372. doi:10.1038/hdy.1994.183
- James, J.W. (1970). The founder effect and response to artificial selection. *Genetical Research*, 16, 241-250. doi:10.1017/S0016672300002500
- Keller, L.F., and Waller, D.M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, 17, 230-241. doi:10.1016/S0169-5347(02)02489-8
- Lande, R. (1995). Mutation and conservation. *Conservation Biology*, 9, 782-791. doi:10.1046/j.1523-1739.1995.09040782.x
- Lerner, I.M. (1954). *Genetic Homeostasis*. Edinburgh: Oliver & Boyd.
- López-fanjul, C., Fernández, A., and Toro, M.A. (1999). The role of epistasis in the increase in the additive genetic variance after population bottlenecks. *Genetical Research*, 73, 45-59. doi:10.1017/S0016672398003619
- Lynch, M., Conery, J., and Burger, R. (1995). Mutation accumulation and the extinction of small populations. *The American Naturalist*, 146, 489-518. doi:10.1086/285812
- Miller, P.S. (1994). Is inbreeding depression more severe in a stressful environment? *Zoo Biology*, 13, 195-208. doi:10.1002/zoo.1430130302
- Miller, P.S., and Hedrick, P.W. (2001). Purging of inbreeding depression and fitness decline in bottlenecked populations of *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 14, 595-601. doi:10.1046/j.1420-9101.2001.00303.x
- Nei, M., Maruyama, T., and Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution*, 29, 1-10. doi:10.1111/j.1558-5646.1975.tb00807.x
- Saccheri, I.J., Brakefield, P.M., and Nichols, R.A. (1996). Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). *Evolution*, 50, 2000-2013. doi:10.1111/j.1558-5646.1996.tb03587.x
- Sved, J.A., and Ayala, F.J. (1970). A population cage test for heterosis in *Drosophila pseudoobscura*. *Genetics*, 66, 97-113. doi:10.1093/genetics/66.1.97
- Templeton, A.R., and Read, B. (1983). Elimination of inbreeding depression in a captive herd of Speke's gazelle. In C.M. Schonewald-Cox, et al. (Eds.), *Genetics and Conservation: a Reference for Managing Wild Animal and Plant Populations* (pp. 241-261). Menlo Park, CA: Benjamin/Cummings Publishings.
- Templeton, A.R., and Read, B. (1984). Factors eliminating inbreeding depression in a captive herd of Speke's gazelle (*Gazella spekei*). *Zoo Biology*, 3, 177-199. doi:10.1002/zoo.1430030302