Inbreeding depression, loss of genetic variation, and survival in a high salt diet.

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Since Darwin (1859), it has been assumed that genetic variation (phenotypic variation for Darwin) is essential for adaptive evolution. This has been shown to be true in *Drosophila melanogaster* experiments where selection response is reduced in inbred lines with no genetic variation compared to outbred lines that have genetic variation (Falconer and Mackay, 1996; Clayton and Roberson 1955; Woodruff and Thompson, 2008). Here we have tested if inbreeding, with a slow loss of genetic variation over time and the associated inbreeding depression caused by the homozygosis of deleterious mutations (Hedrick 2005; Lynch and Walsh, 1998), reduces the potential for populations to adapt to a new stressful environment. Onasch and Woodruff (2008) have shown that inbreeding does cause a significant decrease in viability (progeny numbers) in *D. melanogaster*. This experiment, however, was performed in a non-stressful environment.

In a modification of Frankham *et al*. (1999) and Zhang *et al*. (2011), we have measured the ability of outbred and inbred lines of *D. melanogaster* that were originally isolated from nature to survive and produce progeny on increasing concentrations of dietary NaCl, up to a toxic level (6%). Twelve mated females were captured from nature (Perrysburg, Ohio) on July 30, 2010. From these females, twelve isofemale lines were maintained by sibling matings (a single virgin female and a single sib male were mated in a vial each generation). In addition, three outbred lines (OBA, OBB, and OBC) were set up by mixing the progeny of four isofemale lines per outbred line in generation two and maintaining these lines by mass transfers each generation into bottles. These outbred lines were controls that contained genetic variation.

At generation three, five females and five males from each of the outbred lines were placed in vials with 0% NaCl, 2% NaCl, 4% NaCl, and 6% NaCl (mixed into Carolina Instant Drosophila Food). In addition, five females and five males of each of the seven inbred lines (IBC, IBE, IBI, IBJ, IBM, IBP, and IBU), which had been brother/sister mated each generation, were placed in vials with the same NaCl concentrations. All vials were placed at 25ºC and the parents were removed after seven days. Furthermore, the progeny were counted per vial for a total of 14 days from the day the vials were initiated. The same procedure was repeated for generations six and nine. It was expected that the outbred lines would continue to have genetic variation throughout this study, while the inbred lines, which were maintained each generation by single brother/sister matings, would with time lose genetic variation and show inbreeding depression.

The results (number of progeny per vial) for generation three are shown in Table 1, for generation six in Table 2, and for generation nine in Table 3.

In all generations, 6% NaCl was toxic to the outbred (OB) and inbred (IB) lines; no progeny were recovered in any line. Frankham *et al* (1999) and Zhang *et al* (2011) saw similar results.

Inbreeding caused a significant decrease in the number of progeny in all the concentrations of NaCl, whereas the outbred lines had a reduction in offspring numbers only at 4% NaCl. From unpaired t tests, the outbred (OB) lines had a significant reduction in progeny at 4% NaCl as compared to 0% at generation six ($P = 0.0001$) and at generation nine ($P = 0.03$), but not in generation three ($P = 0.14$). Hence, 4% salt reduces viability even in lines with genetic variation. The outbred lines, however, did not have a significant decrease in offspring in 2% NaCl.

On the other hand, the inbred (IB) lines had a significant decrease in progeny at 2% and 4% saline. In inbred generation three, there was a significant reduction ($P = 0.0007$) in the in 0% *vs*. 4%

NaCl vials. In addition, for the inbred lines there were significant decreases in progeny in 0% *vs*. 2% in generation six (P = 0.018), and generations nine (P = 0.0079), as well as decreases in progeny numbers in 0% *vs*. 4% in generation six (P = 0.0016) and generation nine (P < 0.0001).

Table 1. Progeny numbers after three generations.

Line	0% NaCl	2% NaCl	4% NaCl	6% NaCl
OBA	57	10	14	0
OBB	18	21	29	0
OBC	49	40	2	0
OB Avg.	41.33	23.67	15.00	0
IBC	31	22	14	0
IBE	29	34	2	0
IBI	36	32	2	0
IBJ	18	28	12	0
IBM	38	16	11	0
IBP	14	14	4	0
IBU	19	23	11	0
IB Avg.	26.43	24.14	8.00	0

Table 2. Progeny numbers after six generations.

Line	0% NaCl	2% NaCl	4% NaCl	6% NaCl
OBA	38	51	12	0
OBB	36	49	13	0
OBC	38	20	9	0
OB Avg.	37.33	40.00	11.33	0
IBC	30	17	5	0
IBE	25	18	6	0
IBI	20	5	1	0
IBJ	22	11	3	0
IBM	67	23	2	0
IBP	34	19	2	0
IBU	77	9	8	0
IB Avg.	39.29	14.57	3.86	0

Table 3. Progeny numbers after nine generations.

Line	0% NaCl	2% NaCl	4% NaCl	6% NaCl
OBA	68	47	18	0
OBB	97	36	40	0
OBC	132	75	45	0
OB Avg.	99.00	52.67	34.33	
IBC	46	26	2	0
IBE	33	18	0	0
IBI	59	45	21	0
IBJ	49	14	0	0
IBM	39	21	5	0
IBP	39	27	13	0
IBU	28	5	4	0
IB Avg.	41.81	22.29	6.43	

In conclusion, inbreeding, with loss of genetic variation and inbreeding depression can decrease the ability of populations to adapt to a new stressful environment, such as an increase in the concentration of NaCl in the diet. In this study the inbred lines produced significantly lower numbers of progeny in two and four percent NaCl after three, six, and nine generations. In contrast, the outbred lines, which contained genetic variation, only had a reduction in offspring numbers at 4% NaCl.

Others have also observed that there is an increased sensitivity to stressful environments in inbred lines of *D. melanogaster* and other organisms (Miller, 1994; Armbruster and Reed, 2005). This is one reason why in conservation biology it is so important to avoid reductions in population sizes, which cause inbreeding and could lead to an inability of a population or species to respond to a changing environment, such as new parasites or new environmental stresses (Frankham *et al*., 2002).

Questions that could be asked of students include: 1) how much genetic variation is expected to be lost each generation if the variation is neutral (not deleterious or beneficial)? The amount is 1/(2N) per generation, with N being the effective population size. Discussion of this topic can be found in Hedrick (2005). 2) Is inbreeding depression and the loss of genetic variation a problem for organisms in nature? Yes. See a discussion in Crnokrak and Roff (1999). 3) Is there evidence that inbreeding depression occurs in humans? The answer is yes. Infant mortality is higher in the offspring of matings of first cousins than in matings between unrelated individuals (Freeman and Herron, 2007; Hedrick, 2005).

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Lethal mutations and their elimination by selection in natural populations of *Drosophila melanogaster*.

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Lethal mutations are surprisingly frequent in all organisms. For example, up to 70% of *Drosophila melanogaster* in nature carry at least one recessive lethal mutation (Crow, 1993a,b; Lynch *et al*., 1999; Azad *et al*., 2003), and new lethal mutations arise in about six percent of these flies (Simmons and Crow, 1977; Woodruff *et al*., 1983, 1984, 1996; Fu and Huai, 2003; Gao *et al*., 2011). In humans, recessive mutations that can cause death in homozygotes or hemizygotes (X-linked in males) before reproductive maturity are numerous (Morton, 1981; Strachan and Read, 2004; also see Online Mendelian Inheritance in Man (OMIM) at the National Center for Biotechnology Information at http://www.ncbi.nim.hih.gov.omim). Examples of such mutations in humans include Duchenne muscular dystrophy, Lesch-Nyhan syndrome, congenital erythropoietic porphyria, and cystic fibrosis (Morton, 1981; Cummings, 2009).

In addition, most lethal mutations are not completely recessive (Muller, 1950; Simmons and Crow, 1977; Crow and Simmons, 1983; Crow, 1993a,b; Garcia-Dorado and Caballero, 2000). Organisms that are heterozygous for a recessive lethal mutation (*Ll,* with *l* being the lethal mutant allele) have a fitness that is lower than those with homozygous dominant alleles (*LL*). This can be modeled as follows where *L* is the wild- type allele, *l* is the recessive deleterious mutant allele, *s* is the selection coefficient (with s = 1 for lethals), and *h* is the dominance coefficient.

	For autosomal mutations			For X-linked mutations		
	LL	*Ll*	*ll*	*LL female*	*Ll female*	*lY male*
Fitness =	1	1-*hs*	1- *s*	1	1-*hs*	1-*s*
Fitness =	1	1-*h*	0	1	1-*h*	0

A completely recessive mutant allele would have $h = 0$ in heterozygotes, making the fitness of the heterozygotes the same as the *LL* homozygotes. Most lethal mutations, however, have *h* values greater than zero, *i.e*., they are not completely recessive and the heterozygotes (*Ll*) have a fitness that