How Much Does Inbreeding Reduce Heterozygosity? Empirical Results from *Aedes aegypti*

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Abstract. Deriving strains of mosquitoes with reduced genetic variation is useful, if not necessary, for many genetic studies. Inbreeding is the standard way of achieving this. Full-sib inbreeding the mosquito *Aedes aegypti* for seven generations reduced heterozygosity to 72% of the initial heterozygosity in contrast to the expected 13%. This deviation from expectations is likely due to high frequencies of deleterious recessive alleles that, given the number of markers studied (27,674 single nucleotide polymorphisms [SNPs]), must be quite densely spread in the genome.

For many kinds of genetic studies (e.g., classical mapping, quantitative trait locus [QTL] analyses, genome sequencing), strains with reduced genetic variation (reduced heterozygosity) are desired. In our efforts to obtain an improved complete genome sequence for the mosquito *Aedes aegypti*, we have been attempting to derive more homozygous strains to make assembly easier. We report here one such attempt and show that even very close inbreeding may not reduce heterozygosity nearly as much as theoretically predicted.

In theory, if one crosses single full-sibs (brother-sisters), the offspring should have 75% of the heterozygosity of the parents, i.e., one quarter of variable loci become homozygous each generation on average. Repeating this for n generations should produce offspring with $(0.75)^n$ of the initial heterozygosity.

The work reported here is on a sample of *Aedes aegypti* collected in New Orleans in 2014. Four single male–female matings provided the initial families on which inbreeding was performed. Five full-sib (brother–sister) matings were made from each of these families to produce the first generation (F_1) inbred. Of the 20 matings, 18 produced viable eggs. Four of 18 lines were randomly chosen and five full-sib crosses were made from each. Of these 20 crosses, 14 produced > 10 offspring and four with the most offspring were continued. Multiple crosses were continued in this manner until generation seven when only 3/20 crosses produced eggs, in two cases there were < 10 eggs and in one ~50 eggs. Clearly, inbreeding depression was occurring as indicated by the number of crosses producing viable eggs. The most productive strain was continued and genotyped.

We genotyped offspring from the field-collected eggs ($N = 24$) and the F_7 -inbred strain ($N = 8$) using a SNP chip.¹ All 32 mosquitoes genotyped reliably at 27,674 SNP loci and frequencies of heterozygotes at these loci in each individual were determined. Table 1 reports the average number of SNPs heterozygous per mosquito.

The reduction in heterozygosity observed is much less than theoretically expected. After seven generations of single-sib inbreeding, we expected 13.3% $[(0.75)^7]$ of the initial heterozygosity of 22% or 2.9%, but observed 15.9%.

This result is very likely due to selection each generation for greater than average heterozygosity; only strains that produce fertile offspring were continued. Recall that the expected reduction to 75% heterozygosity of the parents is an average and that there is a distribution of heterozygosity

in the offspring. Almost certainly the most heterozygous in the distribution were being selected. This selection for heterozygotes is also evident in the observed and expected heterozygosity in the F_7 mosquitoes genotyped: 0.159 versus 0.127. The expected is that predicted by Hardy–Weinberg (H–W) for the allele frequencies in the F_7 population, but those sampled (those viable in that generation) exceed the expectations because heterozygotes preferentially survived.

Inbreeding depression due to increasing homozygosity is a well-established phenomenon. In *Aedes* mosquitoes, early work using isozymes (allozymes) to track reduction of heterozygosity during inbreeding produced results similar to those reported here, namely less reduction in heterozygosity and excess of heterozygotes in those mosquitoes surviving inbreeding.^{2–4} Similarly, in a QTL study of *Ae. aegypti* using inbred strains Gomez-Machorro and others found that 22/28 loci deviated from H–W expectations due to an excess of heterozygotes.⁵ These previous studies hinted that recessive deleterious alleles are common in the *Ae. aegypti* genome, but because they were based on the limited number of loci available at that time (up to 40), it was not clear just how dense. This study using tens of thousands of markers reveals just how dense these recessive deleterious alleles must be.

These results suggest it is wise to actually measure reduction in genetic diversity due to inbreeding rather than rely on theoretical expectations. The tools available today to do this are much more accurate and efficient.

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TABLE 1

Observed and expected heterozygosity by Hardy–Weinberg proportions in *Aedes aegypti* sampled directly from the field and after seven generations of single brother–sister inbreeding

Generation	Observed heterozygosity	Expected heterozygosity by Hardy–Weinberg	Theoretical expectation
Field reared	0.220	0.234	
F_7 inbred	0.159	0.127	0.029

Theoretical expectation is that expected on average for this level of inbreeding.

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REFERENCES

1. Evans BR, Gloria-Soria A, Hou L, McBride C, Bonizzoni M, Zhao H, Powell JR, 2015. A multipurpose high-throughput SNP chip for the dengue and yellow fever mosquito, *Aedes aegypti*. *G3 (Bethesda)* 5: 711–718.
2. Matthews TC, Craig GB, 1987. Heterozygosity in inbred strains of the tree-hole mosquito *Aedes triseriatus*. *Biochem Genet* 25: 647–655.
3. Matthews TC, Craig GB, 1989. Isozyme polymorphisms maintained by lethal loci in inbred strains of *Aedes triseriatus*. *J Hered* 80: 53–57.
4. Munstermann LE, 1994. Unexpected consequences of colonization and inbreeding—allozyme training in Culicidae (Diptera). *Ann Entomol Soc Am* 87: 157–164.
5. Gomez-Machorro C, Bennett KE, del Lourdes Munoz M, Black WC, 2004. Quantitative trait loci affecting dengue midgut infection barriers in an advanced intercross line of *Aedes aegypti*. *Insect Mol Biol* 13: 637–648.