Both multiplexes had a low rate of PCR failure (0.07% – 1.4%) and a low mis-scoring error rate of 3.9% (Table 2). Microsatellite 4 (*DROMHC*) showed extreme allelic drop-out under these conditions and thus was excluded from analysis and not scored. Microsatellite 5 (*DROGPAD*) also exhibited evidence of allelic drop-out or null alleles as it had an excess of homozygotes (H_o = 0.071) and 29% of the populations significantly deviated from Hardy-Weinberg expectations (Table 2). Thus microsatellite 5 should be excluded from population genetic analyses. After excluding these two potentially compromised loci, MP1 and MP2 comprise ten microsatellite loci that amplify robustly and provide good but not complete genome coverage. The total number of alleles for the remaining ten loci ranges from 4 – 12 and the mean observed heterozygosity ranged from 0.3 – 0.7 (Table 2). The remaining ten loci adhered to Hardy-Weinberg expectations in 96.6 – 100% of populations, with the mean allele proportions displayed in Figure 1.

We have described a set of optimised PCR reaction conditions that, in conjunction with step-down PCR cycling conditions, result in robust, repeatable and non-preferential amplification of a suite of ten microsatellite markers. Thus this paper provides *Drosophila* researchers with an efficient and cost effective alternative to multiple single PCR reactions.

References: Bercovich, D., Z. Regrev, T. Ratz, A. Luder, Y. Plotsky, and Y. Gruenbaum 1999, BioTechniques 27: 762-770; Caracristi, G., and C. Schlötterer 2003, Molec. Biol. Evol. 20: 792-799; England, P.R., D.A. Briscoe, and R. Frankham 1996, Genet. Res. 67: 285-290; Goldstein, D.B., and A.G. Clark 1995, Nucleic Acids Res. 23: 3382-3386; Harr, B., 2000, Ph.D. Thesis, University of Konstanz Germany; Harr, B., and C. Schlötterer 2000, Genetics 155: 1213-1220; Henegariu, O., N.A. Heerema, S.R. Dlouhy, G.H. Vance, and P.H. Vogt 1997, BioTechniques 23: 504-511; Holleley, C.E., 2007, Dros. Inf. Serv. 90: 137-138; Schlötterer, C., C. Vogl, and D. Tautz 1997, Genetics 146: 309-320; Schug, M.D., T.F.C. Mackay, and C.F. Aquadro 1997, Nat. Genet. 15: 99-102; Schug, M.D., K.A. Wetterstrand, M.S. Gaudette, R.H. Lim, C.M. Hutter, and C.F. Aquadro 1998, Mol. Ecol. 7: 57-70; Swindell, W.R., and J.L. Bouzat 2006, Evolution 60: 1014-1022; Vallone, P.M., and J.M. Butler 2004, BioTechniques 37: 226-231.

An inexpensive and efficient method for obtaining *Drosophila* heads.

Pu, Rongsun,[#] Ailleen Manansala,* Matthew Sorensen,* and Opeyemi Tayo-Balogun*. Department of Biological Sciences, Kean University, Union, NJ 07083. [#]author for correspondence. Tel: (908)737-3663; E-mail: rpu@kean.edu; *undergraduate student authors and equal contributors to this work.

During our investigation of calcium/calmodulin-dependent protein kinase II which is highly concentrated in the heads of *Drosophila melanogaster*, we have devised a method to quickly separate the heads of the flies from the bodies.

The flies are moved into a clear, empty vial with a foam cap. Then small chunks of dry ice are put into the vial. The carbon dioxide immobilizes flies within a few seconds, while the low temperature minimizes protein degradation. The vial is manually shaken for a few minutes until the wing tissue comes off, as evident by the clear specks on the sides of the vial. By this time most heads have snapped off. The contents of the vial (including remaining pieces of dry ice) are then examined under a dissecting microscope and the heads could be easily separated from the bodies using a fine brush or dissecting needle.

Using this method, we were able to obtain about 200 fly heads under a dissecting microscope in less than 5 minutes. This method works well when the air is dry. Otherwise, the moisture from the air would keep the flies mushy and inseparable.

A method to measure associative learning for different size larvae.

Cooper, Richard M., and Robin L. Cooper. Dept. of Biology, University of KY, Lexington, KY. 40506-0225 USA; Email: cooper_richie@yahoo.com

Since the time of Alpatov (1929) people have been interested in studying environmental impacts on the developmental rate of larval fruit flies. Kaznowski *et al.* (1985) demonstrated that the thickness of the cuticle is gradual throughout the instar stages and does not jump with every molt cycle. This study was important since it explained why a gradual development was observed in these molting instars. This also suggests that diet could have an effect on development within an instar stage. Biochemical studies on larval fruit flies showed how the amount of proteins increases with development (Church and Robinson, 1966) and that the nitrogen and phosphate content increased with each molt stage (Watts *et al.,* 2006). These studies suggest that fruit flies are dependent on particular elements. Some factors may be developmentally rate limiting for the whole body. Despite whole larval developmental interest over the years, little attention has been given to dietary regulation on neuronal development of the larval central nervous system (CNS).

The developmental need of the CNS may be more restrictive than the whole body development due to the complexity of factors in formation and maintenance the central nervous system. There are many synapses for the refined communication. So even though whole body size might appear to be normal, one cannot assume the neural functions are fully intact. One approach to examine the effects of diet on proper development of neuronal circuits would be particular behavioral assays. It is apparent that most of the attention given to *Drosophila* behaviors has concentrated on adults, but there are a number of larval behavioral assays established. Eating and locomotion are commonly used larval behaviors (Sewell *et al.* 1975; Neckameyer, 1996; Li *et al.,* 2001). In the last few years, revolutionary studies have shown that larvae have the ability to demonstrate associative learning (Scherer *et al.,* 2003; Gerber *et al.,* 2004; Hendel *et al.,* 2005). However, the assays used are very dependent on the experimental design and might not be best suited for larvae of all developmental stages or sizes that could be altered from developmental retardation.

More direct effects on a neural circuit can also be examined. Recently, neuromodulators, such as serotonin (5-HT) and dopamine, have been shown to have a role on activity of a sensory-CNS-motor circuit in *Drosophila* larvae (Dasari and Cooper, 2004; Dasari *et al.,* 2007) as well as direct effects on the larval heart (Dasari and Cooper, 2006). So one might assume a diet restrictive in essential amino acids that are precursors to 5-HT and dopamine might impact the development in the wiring of the CNS.

In our current studies we used a simple restrictive diet of glucose and water (1 gram for 10 mls of water) and placed newly hatched embryos (1st instar) in this solution and maintained them at room temperature (21^{o}C). With this diet larvae develop very slowly compared to ones fed a standard cornmeal-agar-dextrose-yeast medium diet commonly used for culturing. The larvae would remain within each instar for a longer time and some would take 14 days until beginning to form a pupa. The size of the larvae at each stage is drastically reduced compared to an equivalent instar stage in the controls fed an enriched diet. We determined instar stage based on the numbers of teeth present on the mouth hooks (Strasburger, 1932; Demerec, 1994).