HEXANE PRESERVES BIOLOGICAL ACTIVITY OF ISOZYMES AND DNA

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ABSTRACT. Live or frozen insects are required for using isozyme and DNA RFLP methods in studies on population structure, systematics and incrimination of sibling species. Difficulty in keeping insects alive or unavailability of liquid nitrogen or dry ice at regular intervals during extended collection trips poses a serious problem. We describe a method for preserving insects in hexane, under field conditions, for isozyme and DNA analysis.

Isozyme electrophoresis and analysis of DNA restriction fragment length polymorphism (DNA RFLP) are routinely used in studies involving population genetics, phylogeny and systematics of medically important insects. For conducting isozyme and DNA RFLP studies, live or frozen insects are required. This is an inconvenient constraint in the study of natural populations, because the remote locations where insect samples must be collected limits access to adequate laboratory facilities with freezers that preserve material at −15°C. The collector usually has the choice of transporting live material or using liquid nitrogen or dry ice. For fragile insects (e.g., mosquitoes), transporting live material is not satisfactory, because this often results in high mortality. Liquid nitrogen or dry ice carried in specialized containers can be used for temporary (up to 2 weeks) storage, but for extended collection trips there is usually no readily available source of either coolant in developing countries. Recently, while trying to develop techniques that would allow us to correlate the patterns of cuticular hydrocarbons with allozyme phenotypes, we discovered that hexane can serve as a preservative for enzymes and DNA and could be very useful for storing mosquitoes under field conditions. The activities of allozymes of adults of *Anopheles quadrimaculatus* Say, preserved in hexane for up to 2 months, at different temperatures, were compared with the activity of live samples.

Adult mosquitoes were transferred into three, 1.5-ml Eppendorf tubes (up to 100 mosquitoes per tube) containing hexane and stored at 25°C (room temperature), at 4°C (in refrigerator) or at −15 to −20°C. Samples were removed from each tube at different intervals, and isozyme activity was tested by electrophoresis. The relative activity of each enzyme was compared to specimens stored at −15°C and to mosquitoes that were freshly killed.

Starch-gel electrophoresis was conducted primarily according to Steiner and Joslyn (1979) with a few modifications (Narang et al. 1989a, 1989b). Homogenates of individual mosquitoes (grinding buffer, pH 7.0: 10 mM Tris, 1 mM EDTA, 1 mM 2-mercaptoethanol and 5 mM dithiothreitol) were absorbed onto two, 10 × 2.5 mm wicks (Whatman 3-mm paper), and applied to two, 11-mm thick starch gels (one wick per gel). After electrophoresis, each gel was cut into seven 1.5-mm thick slices, stained for specific enzymes and fixed as soon as bands were of desired intensity (30–90 min). Electromorphs of 22 presumptive loci of 13 enzymes were studied. These included esterases (EST, 5 loci), glutamate oxaloacetate transaminase (GOT, 2 loci), hydroxy acid dehydrogenase (HAD, 1 locus), isocitrate dehydrogenase (IDH, 2 loci), malate dehydrogenase (MDH, 1 locus), malic enzyme (ME, 1 locus), mannose phosphate isomerase (MPI, 2 loci), peptidase (PEP, 2 loci), aconitase (ACON, 1 locus), aldehyde oxidase (AO, 1 locus), hexokinase (HK, 2 loci), phosphoglucose isomerase (PGI, 1 locus) and phosphoglucomutase (PGM, 1 locus). When necessary, a single slice was stained for 3 enzyme systems, such as hydroxy acid dehydrogenase (HAD), malate dehydrogenase (MDH) and malic enzyme (ME) or 2 enzymes, phosphoglucomutase (PGM) and glucose phosphate isomerase (PGI). These multiple enzyme zymograms were easy to score due to differences in migration of their respective electromorphs in the gel.

The scoring system for enzyme activity was strictly subjective and consisted of a numerical scale from 0 to 4, with a rating of 4 being equal to freshly killed mosquitoes and a 0 representing undetectable activity.

After 7 days of storage in hexane, samples from each of the test groups were used for mitochondrial DNA (mtDNA) RFLP according to the method of Cockburn and Seawright (1988).

The effect of storage on the activities of electromorphs of allozyme loci is shown in Table 1. All of the enzymes retained activity in samples stored in hexane for 2 days at room temperature, but after 3 days the activity of the enzymes declined. Good activity was still present for 18

Table 1. Change in relative staining activity of electromorphs of isozyme loci in adults of *Anopheles quadrimaculatus* species A stored in hexane.

	Control (Live adults)	Adults stored in hexane at indicated temperature									
		Days at									
		4° C					Room temp. (25°C)				
Locus	0 days	2	3	7	30	60	2	3	7	30	60
Pgi-1	4	4	4	4	4	3	4	4	2	1	0
Acon-1	4	4	4	4	4	4	2	1	ND	0	0
Hk-1	4	4	4	4	3	3	4	4	3	1	1
Hk-2	4	4	4	4	3	3	4	4	2	1	1
Pep-4	4	4	4	4	3	3	4	4	2	1	1
Me-1	4	4	4	4	3	3	4	4	2	1	0
Had-3	4	4	4	4	2	2	4	4	2	0	0
Pgm-3	4	4	4	3	3	1	1	1	ND	0	0
Est-2	4	4	4	4	3	3	4	4	2	1	0
Est-5	4	4	4	4	2	2	4	4	4	3	2
Est-6	4	4	4	4	1	1	4	4	4	0	0
Est-4	4	4	4	4	0	0	4	3	1	0	0
Mpi-3	4	4	4	3	2	0	4	2	1	0	0
Mpi-1	4	4	4	2	1	1	ND	ND	ND	ND	ND
Got-2	4	4	4	2	1	0	4	2	0	0	0
Mdh-1	4	4	4	2	0	0	4	4	0	0	0
Got-1	4	4	4	1	1	1	2	1	0	0	0
Est-7	4	4	4	0	0	0	2	ND	0	0	0
Pep-2	4	4	4	0	0	0	1	1	0	0	0
Ao-1	4	4	3	2	0	0	2	2	0	0	0
Idh-1	4	3	3	0	0	0	2	0	0	0	0
Idh-2	4	3	3	0	0	0	2	1	0	0	0

4, highest activity; 0, low to undetectable activity; ND, no data available.

enzymes after 7 days in refrigerated samples in hexane, but virtually nil for the remaining four. Many of the enzymes were still very active after storage in a refrigerator for 30 days. When quickly killed mosquitoes were stored in air (at room temperature without hexane), except for ME and PGI, activities of all other enzymes were greatly reduced after 24 h (equivalent to level 1). After 48 h at room temperature, no activity was detectable (greatly reduced for ME and PGI). Similarly, enzymes in refrigerated samples (without hexane) lost activity after 60 h. When samples in hexane were stored at −15°C, the activity was higher than when samples were stored at this temperature range without hexane. These results are relevant under our assay conditions. The mtDNA RFLPs in the test groups were comparable to RFLPs obtained from a control group (live mosquitoes).

Individual mosquito homogenate was loaded in 2 gels. Each gel was cut into 7 slices after electrophoresis. Thus, staining intensities of electrophoretic bands in each gel slice represented the activity in 1/14 of a mosquito. If the mosquito homogenate was loaded into a single starch gel or 1- to 2-mm thick polyacrylamide gel, the relative activities (such as 1 or 0 in Table 1) might be higher. It would therefore be desirable to check the preservative effect of hexane for each insect species under the most favorable conditions for electrophoresis.

The results indicate that hexane can be used for the storage and transport of insect samples for subsequent laboratory studies of isozymes. One additional advantage of using hexane is that the hexane extract can be analyzed for hydrocarbon patterns.

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