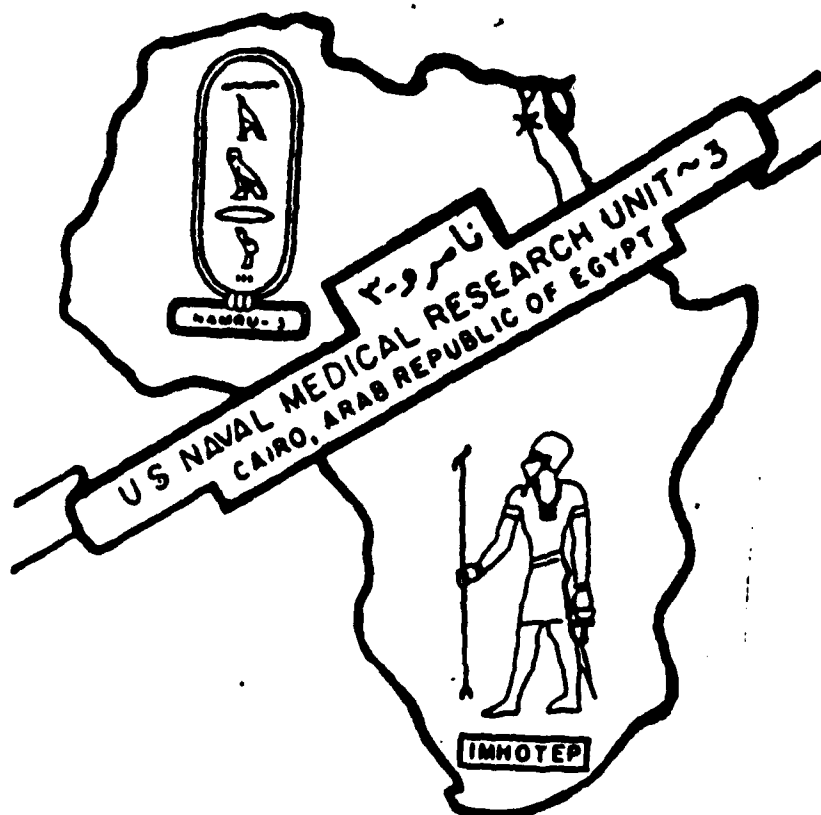
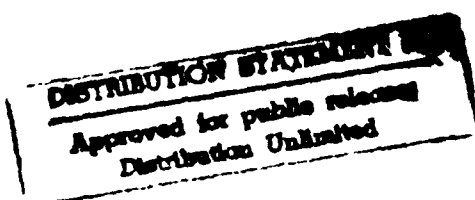


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ENZYME POLYMORPHISM AND GENETIC VARIABILITY OF ONE COLONIZED AND SEVERAL FIELD POPULATIONS OF PHLEBOTOMUS PAPATASI (DIPTERA: PSYCHODIDAE)

BY

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Enzyme Polymorphism and Genetic Variability of One Colonized and Several Field Populations of *Phlebotomus papatasi* (Diptera: Psychodidae)

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ABSTRACT The Alexandria laboratory colony and five field populations of *Phlebotomus papatasi* (Scopoli) from Egypt were analyzed for genetic variation at 17 enzyme loci. The laboratory colony was characterized by a low level of genetic variation as measured by the average number of alleles per locus ($\bar{A} = 1.70 \pm 0.16$) and the average expected heterozygosity ($H_e = 0.06 \pm 0.02$). Polymorphism was observed at 23.5% of the examined loci, and genotype frequencies at two loci (PGM, AK-2) were found to deviate slightly from the Hardy-Weinberg equilibrium. In contrast, the average number of alleles per locus for field populations ranged from $\bar{A} = 2.35 \pm 0.20$ to 2.76 ± 0.10 , and H_e ranged from 0.15 ± 0.03 to 0.21 ± 0.05 . All loci of field populations exhibited polymorphism, ranging from 47.0% to 76.5%, and four to seven loci in each population were found to deviate from the Hardy-Weinberg equilibrium. Deviations in both colonized and field populations were caused by heterozygote deficiency. Despite geographic isolation and some individual deviations from the Hardy-Weinberg equilibrium, no evidence of significant genetic difference was obtained for any of the populations sampled. Calculated indices of genetic distance and genetic identity for the five field populations showed minor variation but were collectively representative of a single, genetically uniform population.

KEY WORDS *Phlebotomus papatasi*, genetic variation, polymorphism

Phlebotomus papatasi (Scopoli) has been recorded in Egypt from habitats ranging from heavily populated urban centers to barren desert. Given the extremely limited flight range of this sand fly (WHO 1984), it is reasonable to consider that Egyptian populations of *P. papatasi* from different habitats and locations may differ genetically. Variable morphological characters of some Egyptian populations of *P. papatasi* have been noted (Schmidt & Schmidt 1962, Lane 1986) and may result from reproductive isolation or ecological partitioning. At one location, 30% of the adult male *P. papatasi* examined were found to have atypical genitalia (Kassem et al. 1988), and *P. papatasi* from the Sinai express some morphological characteristics similar to a closely related species, *Phlebotomus bergeroti* (Parrot) (D.J.F., unpublished data).

Human cases of cutaneous leishmaniasis (CL) caused by *Leishmania major* (Mansour et al. 1987, 1991) have been reported frequently from the sparsely populated North Sinai, but few cases originate along the Mediterranean coast or in the Nile Delta (Morsy et al. 1985, 1991). *P. papatasi*, the recognized vector of *L. major* in Egypt (Wahba et al. 1990), is found commonly in each of these areas. Recent work with *P. papatasi* from Egypt and Israel has shown that genetically stable strains, susceptible and refractory for *L. major* infection, can be selected within 13 generations, and that selection for refractoriness to infection is associated with a shift from polymorphism to monomorphism at certain enzyme loci (Wu 1989, Wu & Tesh 1990a). Because neither susceptible nor refractory traits are dominant (Wu & Tesh 1990b), it is possible that natural populations of this sand fly vary considerably in their vector competence. Regional differences in this species' ability to develop and transmit pathogens may account for patterns of human disease caused by sand fly-borne pathogens in Egypt.

Successful maintenance of laboratory colonies has been essential to biological research on sand flies (Killick-Kendrick 1978). Laboratory colonies of insects often are accepted as being representative of field populations from which they have been derived, but this may not be a valid

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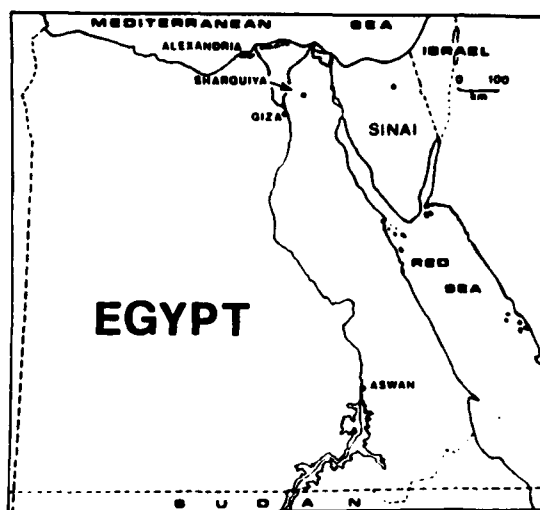


Fig. 1. Map of Egypt showing sampling locations for *P. papatasi*.

assumption, because the colony frequently incorporates only a fraction of the original populations' genetic variability (Lorenz et al. 1984). In addition, colonization, which generally reduces natural genetic variability, may select for certain traits which are rare or normally suppressed in field populations (Lewontin 1974, Tabachnick 1990).

The present report uses enzyme electrophoresis to compare the genetic similarity of field and colonized populations of *P. papatasi*. Our specific objectives were to determine whether genetic differentiation has occurred among field populations of *P. papatasi* and to compare levels of genetic variation of a laboratory colony with those of field populations.

Materials and Methods

Sand Flies. Sand flies were collected during the summers of 1988 and 1989 from five geographically distinct locations, representative of Egyptian sand fly habitats (Fig. 1). These habitats included agricultural areas along the Nile valley in Aswan and Sharquiya; barren desert in the Sinai peninsula; a moist, littoral zone in Alexandria; and an urban locality in Giza Governorate (greater Cairo). Sand flies were collected by CDC light traps, aspiration from inside houses, or by human landing collections, depending on the abundance of flies in each locality. Human cases of cutaneous or visceral leishmaniasis have been reported from each location. Sand flies from each locality were either transferred to the laboratory alive or frozen immediately in liquid nitrogen. Collections were stored at -70°C until they were tested.

The laboratory colony of *P. papatasi* originated from sand flies collected in October 1981 at a

focus of visceral leishmaniasis near Alexandria (Tewfik et al. 1983). This colony was established from 30 ovipositing females and has been maintained for 34 successive generations using the methods described by Schmidt (1964).

Electrophoretic Techniques. Procedures for separation and characterization of sand fly enzymes on cellulose acetate membranes (Helena Laboratories, Beaumont, TX) have been described in detail by Kassem et al. (1990). The following 13 enzymes were selected because they provided clearly interpretable isoenzyme phenotypes: Malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40), isocitrate dehydrogenase (ICD, E.C. 1.1.1.42), 6-phosphogluconate dehydrogenase (6-PGDH, E.C. 1.1.1.43), glucose-6-phosphate dehydrogenase (G-6PDH, E.C. 1.1.1.44), xanthine dehydrogenase (XDH, E.C. 1.2.1.37), adenylate kinase (AK, E.C. 2.7.4.3), hexokinase (HK, E.C. 2.7.1.1), phosphoglucomutase (PGM, E.C. 5.4.2.2), acid phosphatase (ACP, E.C. 3.1.3.2), fumarate hydratase (FUM, E.C. 4.2.1.2), glucose phosphate isomerase (GPI, E.C. 5.3.1.8), and mannose phosphate isomerase (MPI, E.C. 5.3.1.9).

Only individually identified *P. papatasi* were tested. In each population, the most frequent allomorph (allele) at a locus was assigned a value of 100. All other allomorphs were given mobility values relative to their migrating distance to the common 100 position.

Statistical Analysis. Genetic variability of each population was assessed from the number of allomorphs observed at each locus, the proportion of polymorphic loci, observed and expected heterozygosities, and deviation from the Hardy-Weinberg expectations. A locus was considered polymorphic when the frequency of the most common allele was no greater than 0.95 (Ayala et al. 1972). Yates correction was applied for allomorphs observed at low frequency. The statistical significance of gene frequency differences was tested using $R \times C$ tests of independence using G statistics (Sokal & Rohlf 1981). Mean numbers of alleles per locus were compared using one-way analysis of variance (ANOVA) (Sokal & Rohlf 1981). Differences within and between populations for expected heterozygosities were compared nonparametrically by Kruskal-Wallis and Tukey tests (Zar 1984). The inbreeding coefficient (F) in the laboratory colony was estimated by using the following equation:

$$F(t) = 1 - [1 - (1/2n)]^{t-1}$$

where F is the breeding coefficient, t is the number of generations, and n is the size of the breeding population. In an open and randomly mating population with two alleles having frequencies of p and q , the frequency of heterozygotes is $2pq$. However, in closed and inbred laboratory colonies with a coefficient of inbreeding (F), the fre-

Table 1. Genetic variability in Egyptian populations of *P. papatasi*

Population	$\bar{A} \pm SE^a$	$\bar{H}_o \pm SE^b$	$\bar{H}_e \pm SE^c$	P^d
Aswan	2.58 $\pm$ 0.19	0.10 $\pm$ 0.03	0.19 $\pm$ 0.03	76.5
Sinai	2.47 $\pm$ 0.22	0.09 $\pm$ 0.03	0.17 $\pm$ 0.04	47.05
Alexandria	2.47 $\pm$ 0.22	0.09 $\pm$ 0.04	0.21 $\pm$ 0.05	52.94
Sharquiya	2.35 $\pm$ 0.20	0.07 $\pm$ 0.03	0.15 $\pm$ 0.03	64.7
Giza	2.76 $\pm$ 0.10	0.07 $\pm$ 0.03	0.17 $\pm$ 0.03	64.7
Species avg	2.52 $\pm$ 0.06	0.08 $\pm$ 0.01	0.18 $\pm$ 0.01	61.18
Laboratory	1.70 $\pm$ 0.16	0.08 $\pm$ 0.03	0.06 $\pm$ 0.02	23.5

^a Mean number of alleles per locus.^b Mean observed heterozygosity per locus.^c Mean expected heterozygosity per locus.^d Rate of polymorphism (frequency of most common allele ≤ 0.95).

quency of heterozygotes will be reduced by a fraction, F , of their total (Ayala 1982). The genotypic frequencies in the laboratory colony therefore become:

Genotype:	AA	Aa	aa
Frequency:	$p^2 + pqF$	$2pq - 2pqF$	$q^2 + pqF$

Genetic divergence among field populations was estimated through the indices of genetic identity (I) and genetic distance (D) (Nei 1972).

Results

Field Populations. Overall populations, 17 loci were recognized by electrophoretic mobility and pattern. Sample size, gene frequencies, expected heterozygosities, and χ^2 tests for departure from the Hardy-Weinberg equilibrium are presented in Appendix 1. Mean values for the number of allomorphs per locus, heterozygosity per locus, and rate of polymorphism have been summarized by population in Table 1. Using the standard 0.95 criterion for polymorphism, two loci (MDH-2, ICD-2) were consistently monomorphic, whereas five loci (6-PGDH, XDH, PGM, GPI, and FUM) were consistently polymorphic among populations. Enzyme locus polymorphism ranged from 47% in Sinai to 76% in Aswan and collectively averaged 61% for the five field populations. These populations contained one to four allomorphs per locus and averaged 2.35 ± 0.20 to 2.76 ± 0.10 allomorphs per locus. Most of the allomorphs were common to all five populations; however, the Alexandria population expressed a low frequency of the unique variant, GPI 125. The $R \times C$ test revealed that gene frequency distributions were independent of populations (G value = 0.08 - 1.26).

Among the 73 population-locus combinations with polymorphism, 30 cases of significant deviation from the Hardy-Weinberg equilibrium (χ^2 test, $P < 0.05$) were determined. These deviations from equilibrium occurred at 12 of the 17 loci examined, and were variable within each population. Four populations differed from equilibrium at the G6-PDH and XDH loci, and three populations differed at the MDH-1, ICD-1, 6-PGDH, ACP, and FUM loci. MDH-2, AK-2 deviated in two instances, and ICD-2, AK-1, and ME-1 deviated once. These deviations characterized from 29.4% (Alexandria) to 41.2% (Aswan) of the loci in each population. Comparison of observed and expected genotypic ratios revealed that heterozygote deficiency was responsible for 25 of the 26 cases of disequilibrium. Kruskal-Wallis test results showed that expected heterozygosities among the five field populations were not significantly different ($\chi^2_{0.05} [4] = 0.803$). However, inclusion of the laboratory colony data altered the test results to indicate a highly significant difference in expected heterozygosities among the six populations ($\chi^2_{0.05} [5] = 279.27$).

Values of genetic distance (D) and genetic identity (I) are presented in Table 2. Genetic distance between any of the populations was slight, averaging 0.0072. Genetic identity values, each >0.97 for the different populations, also denoted strong genetic similarity existed among these geographically disjunct populations.

Laboratory Colony. The coefficient of inbreeding (F) in the laboratory population of *P. papatasi* was calculated to be 0.432 after 34 generations. This value represented the probability of two gametes uniting to form a zygote carrying alleles

Table 2. Nei's genetic distance (D , above diagonal) and genetic identities (I , below diagonal) among Egyptian Populations of *P. papatasi*

Population	Aswan	Sinai	Alexandria	Sharquiya	Giza
Aswan	—	0.0061	0.0048	0.0076	0.0085
Sinai	0.9939	—	0.0057	0.0168	0.0133
Alexandria	0.9951	0.9942	—	0.0077	0.0044
Sharquiya	0.9923	0.9832	0.9922	—	0.0043
Giza	0.9914	0.9867	0.9955	0.9957	—

identical to those of the parents and was incorporated into determinations of genotype frequency for the colony.

When the laboratory colony (Alexandria strain) was compared with the Alexandria field population (Table 1; Appendix 1), the laboratory colony maintained significantly (one-way ANOVA, $F = 7.31$; $df = 1, 32$; $P = 0.01$) lower levels of genetic variability as determined by polymorphism (Lab $P = 23.5\%$; field $P = 53\%$) and the mean number of allomorphs expressed (lab $\bar{A} = 1.7 \pm 0.16$; field $\bar{A} = 2.47 \pm 0.22$). Mean expected heterozygosity (H_e) of the laboratory colony was calculated to be only 0.06 ± 0.02 , whereas that of the field population was 0.17 ± 0.04 . By Tukey test, the H_e of the laboratory colony was significantly different from the five field populations. In spite of 34 generations of inbreeding, the laboratory population demonstrated a statistically significant departure from the Hardy-Weinberg equilibrium at the PGM and AK-2 loci because of heterozygote deficiency. Gene frequencies for PGM were at the Hardy-Weinberg equilibrium in all five field populations that were sampled. There were no statistically significant differences in observed gene frequencies between the laboratory and field populations for any locus ($R \times C$ test, $P < 0.05$).

Discussion

The most direct and simple expressions of genetic variation in a population are the polymorphism and heterozygosity that are observed at enzyme loci. The Alexandria laboratory colony of *P. papatasi* was polymorphic at only 24% of these loci compared with an average polymorphism of 61% in field populations. High levels of enzyme polymorphism have been reported for both Old World (Mery et al. 1982) and New World sand flies (Caillard et al. 1986), but these have been associated with high levels of both observed and expected heterozygosity. Based upon the observed heterozygosities in our study, field populations of *P. papatasi* from Egypt exhibited low levels of genetic variability. However, the expected heterozygosity calculated for each population was considerably higher than those recorded for most other insect species (Tabachnick & Powell 1979, Tabachnick 1990) and indicated a very high level of genetic variability at each location.

Field populations were surprisingly deficient in heterozygotes at enzyme loci, and the discrepancies between the low observed and high expected heterozygosities resulted in many deviations from the Hardy-Weinberg equilibrium. Significant departures from the equilibrium genotypic frequency predicted by the Hardy-Weinberg law usually have been attributed to natural evolutionary processes such as mutation, migration, drift, and selection, or to a situation in

which individuals within the population are not mating randomly (Ayala 1982). In all but one case, deviations from equilibrium in the *P. papatasi* populations resulted from heterozygote deficiency (or its reciprocal, the excess of rare alleles as homozygotes). A Wahlund effect (Makela & Richardson 1977), because of either the inclusion of *P. papatasi* subpopulations or a cryptic, unrecognized species in the samples, is presumed to be the cause of these heterozygote deficiencies. When the populations were divided into genetically different classes based on those loci with the largest deficiencies of heterozygotes, all loci were found to be in the Hardy-Weinberg equilibrium. This result could support the hypothesis (idea) of a Wahlund effect because of the presence of at least two cryptic species. However, we believe that heterozygote deficiencies arose from sampling during which subpopulations were pooled and treated as if they collectively represented a single *P. papatasi* population.

Genetic homogeneity among populations is indicated from the values of genetic identity and distance calculated for field populations of *P. papatasi* in Egypt. However, to assume that this homogeneity results from extensive gene flow among the five populations is probably incorrect. The close genetic relationship between the distant and isolated locations selected was surprising but was consistent with the absence of any alleles that could be considered unique to a population. The homogeneity of enzyme loci among the field populations of *P. papatasi* indicated that similarities in vector competence also may characterize each population. Previous studies have shown that certain traits of medical importance in sand flies and mosquitoes have been associated with enzyme expression (Tabachnick et al. 1985, Wu 1989). The enzymes are not assumed to be directly responsible for the trait, but the position of their loci may be sufficiently proximal to genes controlling the trait to provide a reliable and easily identifiable marker.

Elimination of rare or uncommon alleles and a resulting decrease in genetic variability has been a frequently reported consequence of laboratory colonization of insect vectors (Munsterman 1979, Lorenz et al. 1984, Tabachnick et al. 1985). Polymorphism and allomorph expression usually are reduced from the original field population. Uncommon alleles present in the field population have been eliminated, and the dominant alleles of the original colony founders have been fixed homozygotically after 34 generations. The end effect of this colonization process has been to restore genotypic frequencies of the population to equilibrium, but at the loss of genetic variability. Disequilibrium did occur at two actively segregating enzyme loci in the laboratory population, but their departure from Hardy-Weinberg equilibrium was not strong, and both loci had

been at or near equilibrium in each of the field populations. The low levels of genetic variability observed in field populations of *P. papatasi* and the further loss of this genetic variability as a result of colonization should be considered in the establishment of laboratory colonies or when making inferences concerning field populations of this sand fly species.

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Appendix 1

Gene frequencies in Egyptian populations of *P. papatasi*

Locus	Allele ^b	Population ^a					
		ASW	SIN	ALX	SHQ	GIZ	LAB
MDH-1	N	104	104	104	80	77	41
	80	0.05	0.02	0.00	0.00	0.03	0.00
	100	0.77	0.58	0.85	0.99	0.96	0.99
	240	0.18	0.40	0.15	0.01	0.01	0.01
	He	0.37	0.48	0.26	0.02	0.08	0.01
	X ²	26.96*	71.22*	31.46*	0.23	0.31	1.02
MDH-2	N	104	104	104	80	77	41
	88	0.00	0.00	0.00	0.01	0.02	0.00
	100	1.00	1.00	1.00	0.99	0.98	1.00
	He	0.00	0.00	0.00	0.02	0.04	0.00
	X ²	0.00	0.00	0.00	25.59*	8.69*	0.00
ME-1	N	115	115	104	80	77	117
	95	0.01	0.00	0.02	0.00	0.01	0.01
	100	0.94	0.80	0.96	1.00	0.95	0.99
	110	0.05	0.20	0.02	0.00	0.04	0.00
	He	0.12	0.32	0.80	0.00	0.01	0.01
	X ²	2.22	86.63*	2.27	0.00	2.10	1.47
ME-2	N	115	115	104	80	77	117
	91	0.00	0.00	0.00	0.00	0.05	0.00
	100	1.00	1.00	1.00	1.00	0.94	1.00
	110	0.00	0.00	0.00	0.00	0.01	0.00
	He	0.00	0.00	0.00	0.00	0.11	0.00
	X ²	0.00	0.00	0.00	0.00	1.14	0.00
ICD-1	N	116	116	104	80	80	30
	71	0.01	0.01	0.00	0.00	0.00	0.00
	100	0.92	0.96	0.99	0.91	0.94	0.82
	128	0.07	0.03	0.01	0.09	0.06	0.18
	He	0.15	0.07	0.01	0.16	0.11	0.17
	X ²	4.47*	0.19	0.00	76.99*	44.42*	3.41
ICD-2	N	116	116	104	80	80	30
	87	0.01	0.01	0.03	0.01	0.01	0.03
	100	0.98	0.99	0.97	0.99	0.99	0.97
	115	0.01	0.00	0.00	0.00	0.00	0.00
	He	0.04	0.03	0.06	0.02	0.02	0.03
	X ²	2.62	0.02	0.09	0.12	31.83*	1.43
6-PGDH	N	97	123	102	80	80	178
	93	0.10	0.03	0.03	0.18	0.03	0.00
	100	0.85	0.93	0.91	0.77	0.94	0.99
	108	0.05	0.04	0.06	0.05	0.03	0.01
	He	0.36	0.28	0.17	0.37	0.12	0.01
	X ²	7.62*	6.94*	3.66	165.80*	1.86	0.73
G6-PDH	N	101	80	97	84	74	24
	83	0.09	0.00	0.05	0.05	0.11	0.00
	100	0.88	1.00	0.90	0.86	0.84	0.96
	129	0.03	0.00	0.00	0.05	0.05	0.04
	He	0.21	0.00	0.18	0.18	0.27	0.04
	X ²	4.65*	0.00	4.89*	5.17*	5.44*	1.48
NDH	N	96	80	112	80	77	48
	86	0.11	0.10	0.13	0.07	0.17	0.00
	100	0.83	0.84	0.81	0.86	0.79	1.00
	113	0.06	0.06	0.06	0.07	0.04	0.00
	He	0.29	0.28	0.32	0.25	0.33	0.00
	X ²	90.94*	5.96*	103.75*	3.06	75.54*	0.00

Appendix continued

Locus	Allele ^b	Population ^a					
		ASW	SIN	ALX	SHQ	GIZ	LAB
AK-1	N	80	80	104	60	79	30
	83	0.00	0.03	0.00	0.00	0.05	0.00
	100	1.00	0.94	1.00	0.93	0.94	1.00
	116	0.00	0.03	0.00	0.07	0.01	0.00
	He	0.00	0.11	0.00	0.13	0.11	0.00
AK-2	X ²	0.00	2.32	0.00	6.73*	1.29	0.00
	N	80	80	104	40	66	30
	88	0.04	0.01	0.04	0.05	0.04	0.05
	100	0.77	0.98	0.96	0.91	0.96	0.80
	108	0.19	0.01	0.00	0.04	0.00	0.15
HK	He	0.37	0.04	0.08	0.17	0.08	0.20
	X ²	6.50*	0.35	82.33*	0.36	2.18	11.40*
	N	111	112	116	80	77	34
	86	0.04	0.01	0.00	0.00	0.01	0.00
	100	0.95	0.97	0.95	1.00	0.97	1.00
PGM	113	0.01	0.02	0.05	0.00	0.02	0.00
	He	0.10	0.06	0.10	0.00	0.06	0.00
	X ²	3.38	0.12	0.36	0.00	0.24	0.00
	N	88	88	95	80	80	40
	90	0.05	0.04	0.11	0.14	0.11	0.05
GFI	100	0.74	0.65	0.60	0.58	0.56	0.41
	114	0.21	0.30	0.27	0.26	0.33	0.54
	126	0.00	0.01	0.02	0.02	0.00	0.00
	He	0.40	0.48	0.58	0.56	0.56	0.31
	X ²	1.00	1.23	5.70	4.39	6.14	10.96*
MPI	N	124	124	104	80	80	60
	81	0.02	0.03	0.03	0.00	0.01	0.00
	100	0.75	0.69	0.62	0.86	0.80	0.79
	118	0.23	0.28	0.31	0.14	0.19	0.21
	125	0.00	0.00	0.04	0.00	0.00	0.00
ACP	He	0.38	0.44	0.62	0.24	0.32	0.19
	X ²	3.64	4.69	7.70	0.21	0.26	0.02
	N	104	96	113	78	80	60
	90	0.01	0.01	0.01	0.01	0.01	0.00
	100	0.92	0.98	0.97	0.94	0.96	1.00
FUM	105	0.07	0.01	0.02	0.05	0.03	0.00
	He	0.15	0.02	0.06	0.11	0.08	0.00
	X ²	1.42	0.01	2.12	1.21	1.60	0.00
	N	104	111	106	80	77	32
	86	0.00	0.01	0.03	0.04	0.02	0.02
FUM	100	0.93	0.96	0.95	0.87	0.89	0.98
	110	0.07	0.03	0.02	0.09	0.09	0.00
	He	0.13	0.07	0.10	0.23	0.20	0.02
	X ²	96.86*	0.63	3.03	6.21*	4.03*	0.39
	N	96	88	98	80	85	122
FUM	87	0.08	0.06	0.06	0.05	0.09	0.00
	100	0.90	0.89	0.82	0.90	0.81	1.00
	125	0.02	0.05	0.12	0.05	0.10	0.00
	He	0.18	0.20	0.31	0.18	0.32	0.00
	X ²	5.65	1.93*	55.43*	2.25	7.22*	0.00

^a ASW, Aswan; SIN, Sinar; ALX, Alexandria; SHQ, Sharquya; GIZ, Giza; LAB, laboratory colony; *, populations not in equilibrium.

^b N, number of individuals in each sample; He, expected heterozygosity; X², deviation from the Hardy-Weinberg equilibrium.

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