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**ABSTRACT** Genetic variation in stable fly, *Stomoxys calcitrans (L.),* populations from Nebraska, Canada, and Texas was sampled. Four of 12 allozyme loci were polymorphic, with an average of 1.7 alleles per locus. Observed and expected heterozygosities were 0.086 and 0.070, respectively. Nei's genetic distance between populations averaged 0.001 and ranged from 0.000 to 0.005. Wright's F statistics revealed greater variation within than among populations. Allele frequencies were homogeneous among temporal samples from a single population. Polymerase chain reaction—restriction fragment length polymorphism (PCR—RFLP) analysis of 6.4 kb of the mitochondrial DNA genome with 16 restriction enzymes revealed no variation in stable fly populations from Canada, Nebraska, and Texas. PCR—RFLP analysis of a 2.0-kb fragment of the nuclear ribosomal DNA internally transcribed spacer region also revealed no variation. The lack of genetic differentiation among stable fly populations indicates high levels of gene How among populations. The low levels of variation observed with biochemical and molecular techniques are consistent with a genetic bottleneck during stable fly colonization of North America.

**KEY WORDS** *Stomoxys calcitrans,* population genetics, isozymes, polymerase chain reaction—restriction fragment length polymorphism

THE STABLE FLY, *Stomoxys* calcitrans (L.), is a widespread, economically important pest of man and animals (Campbell and Hermanussen 1971, Christensen 1982). This species is cosmopolitan, but most abundant in temperate zones ( 1985), where dense populations are associated with confined livestock (Hoffman 1968). Stable fly biting activity reduces weight gain and feed efficiency in cattle (Estienne et al. 1991, Weiman et al. 1992). In addition, stable fly is an important pest of humans at the expanding rural—urban interface (Thomas 1993). Stable fly control technologies, including sanitation and insecticides, are generally unsatisfactory (Hoffman 1968) and knowledge of components of stable fly biology needed for the development of new control strategies is inadequate. Information on overwintering populations (Berry et al. 1978, Berkebile et al. 1994) and stable fly dispersal (Jones et al. 1987) is insufficient for modeling population dynamics and evaluating control strategies for this insect.

Previous studies (Jones et al. 1991, Krafsur 1993) on stable fly population genetics have measured allozyme variation in geographically dispersed populations. The purpose of our study was to examine genetic variation of stable fly in local and dispersed geographic populations using allozymes and to correlate allozyme variation with DNA markers. A nested sampling structure was used to compare variation within and among geographically adjacent and distant populations. This was the 1st effort to compare allozyme, mtDNA, and nuclear rDNA variation of a muscid species within and among the same populations.

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## Methods and Materials

**Stable Flies.** Stable flies were collected from stanchioned calves (Thomas et al. 1989) or by sweep-netting from vegetation and sunny surfaces. Flies were collected from 4 feedlots within 40 km of Lincoln, NE; 3 feedlots within 35 km of Grand Island, NE (130 km west of Lincoln); 1 feedlot at Elgin, NE (150 km north of Lincoln); and from Buffalo Point, MB, Canada (1,500 km north of Lincoln) (Table 1). One of the Lincoln area feedlots (Panama, NE) was sampled 3 times in 1992 and once in 1993 to examine temporal variation. Flies were also examined from the Kerrville laboratory colony (maintained at the USDA—ARS Livestock Insects Research Laboratory, University of Nebraska at Lincoln and derived from a colony at the Agricultural Research Service, USDA Livestock Insects Laboratory Kerrville, TX, which was collected from near Kerrville, TX in 1952 and nev-

**Table 1. Sampling locations and dates for collections of stable fly**

| Sampling location | Abbreviation | Locality, state | Collection date |
|---|---|---|---|
| Seward, Seward County | SEW | Lincoln, NE | 26 July 1992 |
| Hickman, Lancaster County | HIC | Lincoln, NE | 21 June 1992 |
| Valparaiso, Saunders County | VAL | Lincoln, NE | 12 July 1992 |
| Panama, Lancaster County | | Lincoln, NE | 28 June 1992 |
| Panama, Lancaster County | PA2 | Lincoln, NE | 12 July 1992 |
| Panama, Lancaster County | PA3 | Lincoln, NE | 9 Aug. 1992 |
| Panama, Lancaster County | PA4 | Lincoln, NE | 19 Aug. 1992 |
| Grand Island, Hall County | GRI | Grand Island, NE | 9 Aug. 1992 |
| St. Paul, Howard County | STP | Grand Island, NE | 20 Aug. 1993 |
| Dannebrog, Howard County | DAN | Grand Island, NE | 20 Aug. 1993 |
| Elgin, Antelope County | ELG | Norfolk, NE | 15 Aug. 1993 |
| USDA–ARS, Kerr County | KER | Kerrville, TX | 2 May 1993 |
| Buffalo Point, MB | BPT1 | Canada | 19 July 1993 |
| Buffalo Point, MB | BPT2 | Canada | 11 Aug. 1994 |

[a] Laboratory colony established in 1952.

er augmented; S. Kunz, personal communication). Flies were preserved at -80°C, except for the 1994 Buffalo Point, MB, sample (BPT2), which was preserved in 70% ethanol. Voucher specimens were deposited in the University of Nebraska State Museum, Lincoln, NE.

**Allozyme Analysis.** Fifty to 75 flies from each collection (except BPT2) and an equal number of each sex were analyzed. Sex of flies was determined and their heads removed for future molecular analyses. Decapitated bodies were homogenized in a 1.5-ml eppendorf tube with 80 μl of

**Table 2. Thirty-four stable fly enzyme systems screened and 10 systems used in this study**

| Abbreviation[a] | Enzyme | E.C. no.[b] | Loci no. | Buffer |
|---|---|---|---|---|
| AAT | Aspartate aminotransferase | 2.6.1.1 | 2 | TBE |
| ACPH[c] | Acid phosphatase | 3.1.3.2 | 1 | TBE |
| ACOHd | Aconitase hydratase | 4.2.1.3 | 1 | TC |
| ADH | Alcohol dehydrogenase | 1.1.1.1 | — | TC |
| ALPH | Alkaline phosphatase | 3.1.3.1 | — | TBE |
| AO[e] | Aldehyde oxidase | 1.2.3.1 | 1 | TBE |
| CAP | Cytosol aminopeptidase | 3.4.11.1 | 2 | TBE |
| CAT | Catalase | 1.11.1.6 | — | TBE |
| DDH | Dihydrolipoamide dehydrogenase | 1.8.1.4 | 2 | TC |
| EST[c] | Esterase | 3.1.1.1 | 6 | TC |
| FBA | Fructose-biphosphate aldolase | 4.1.2.13 | 1 | TC |
| FBP | Fructose-biphosphate | 3.1.3.11 | — | TC |
| FDH | Formaldehyde dehydrogenase | 1.2.1.1 | 2 | TBE |
| FUMH | Fumarate hydratase | 4.2.1.2 | 1 | TC |
| GCD | Glycerol dehydrogenase | 1.1.1.72 | 1 | TC |
| GO | Glucose oxidase | 1.1.3.4 | — | TBE |
| GDH | Glucose dehydrogenase | 1.1.1.118 | — | TC |
| G3PDH | Glycerol-3-phosphate dehydrogenase | 1.1.1.8 | 1 | TC |
| G6PDH | Glucose-6-phosphate dehydrogenase | 1.1.1.49 | 1 | TBE |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase | 1.2.1.12 | 1 | TC |
| HADH | 3-Hydroxy butyrate dehydrogenase | 1.1.1.30 | 1 | TBE |
| HK | Hexokinase | 2.7.1.1 | 3 | TBE |
| IDH | Isocitrate dehydrogenase | 1.1.1.42 | 1 | TC |
| LDH | L-Lactate dehydrogenase | 1.1.1.27 | 1 | TBE |
| MDH | Malate dehydrogenase | 1.1.1.37 | 1 | TC |
| MDHP | Malate dehydrogenase (NADP+) | 1.1.1.40 | 1 | TBE |
| MPI | Mannose-6-phosphate isomerase | 5.3.1.8 | 1 | TBE |
| PGDH | Phosphogluconate dehydrogenase | 1.1.1.44 | 1 | TC |
| PGI | Phosphoglucoisomerase | 5.3.1.9 | 1 | TBE |
| PGM | Phosphoglucomutase | 5.4.2.2 | 1 | TBE |
| SOD | Superoxide dismutase | 1.15.1.1 | 1 | TBE |
| TPI | Triose-phosphate isomerase | 5.3.1.1 | 1 | TBE |
| THE | Trehalase | 3.2.1.28 | 4 | TBE |
| XDH | Xanthine dehydrogenase | 1.1.1.204 | 1 | TBE |

—, No interpretable staining.
[a] Abbreviations from Murphy et al. (1990).
b International Union of Biochemistry (1984).
Used by Krafsur (1993).
[d] Used for study.
e Used by Jones et al. (1991).

grinding buffer (10% sucrose, 0.01% bromophenol blue, 0.1% Triton X-100 dissolved in Tris-citrate electrode buffer diluted 1:5). One or 2 μl of homogenate was used for each enzyme system.

Polyacrylamide gels (6%) were made following methods of Black and Krafsur (1985). Electrophoresis equipment and methods were similar to Taylor and Peterson (1994). Stain recipes were derived from Munstermann (1979, 1980). After staining, gels were scored, fixed and preserved on cellulose membrane sheets using a Model 583 gel drier (Bio-Rad, Hercules, CA). Alleles for each locus were identified by their migration distances from the origin (Rf) relative to the most frequent allele (designated as 100) in the Kerrville laboratory colony. Thirty-four enzyme systems were screened (Table 2). Ten enzyme systems were selected for the study based on reliability of staining and interpretation of banding patterns in terms of Mendelian characters.

**Polymerase Chain Reaction-Restriction Fragment Length Polymorphism.** DNA extraction, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and restriction digest techniques were similar to Taylor et al. (1996). Four amplicons were analyzed representing ' 42% of the mitochondrial genome (Table 3). Amplicon I was 2.4 kb long and included portions of cytochrome oxidase (CO) I and 1I genes; amplicon 1I was 2.3 kb long and included portions of CO I1 and CO 1I1 genes; amplicon 1I1 was 1.5 kb long and included portions of CO I1I and nicotinamide adenine dehydrogenase (NADH) 5 genes; and amplicon IV was 257 bp long and included a portion of the NADH 4 gene. Primers rDNA1 and rDNA2.144 were used to amplify a portion of the nuclear rDNA gene family (Table 3). The 2-kb amplicon included a portion of the 18S and 28S rDNA genes, internally transcribed spacers (ITS) 1 and 2, and the entire 5.8S rDNA gene. Fourteen to 16 restriction enzymes (New England Biolabs, Beverly, MA) were screened on all mtDNA amplicons and 14 restriction enzymes were used for the rDNA amplicon (Table 4).

Five flies each from BPT1, BPT2, PA3, KER, and ELG (Table 1) were used for the mtDNA RFLP analysis. Two individuals from BPT2, PA3, and KER were used for the nuclear rDNA RFLP analysis.

**Statistical Methods.** Allozyme loci were considered polymorphic when the most common allele had a frequency <0.95 in each population. BIOSYS-1 (Swofford and Selander 1981) was used to calculate expected heterozygosity, $H_e$, Wright's inbreeding coefficient, Wright's $F$ statistic, and Nei's (1978) unbiased minimum distance, D. Wright's (1978) hierarchical analysis of breeding structure for a subdivided population ($F$ statistics) was used to identify sources of spatial differentiation in gene frequencies. Significant deviation from 0 of Wright's $F_{ST}$ and $F_{IT}$ statistics was determined by

**Table 3. Polymerase chain reaction primers**

| | Sequence 5′–3′ | Reverse primer | Sequence 5′–3′ | |
|---|---|---|---|---|
| | TACAATTTATCGCCTAAACTTCAGCC | Sp-4[b] | | 1435–3793 |
| | GGTCAATGTTCATAAATTTGTGG | N3R2[c] | | 3662–5942 |
| | CCTTTTGAATGTGGATTTCATCC | N5F3[c] | | 5725–7329 |
| | GTAGGAGGAGCTGCTATATTAG | N4F3[c] | | 8481–8737 |
| | TTTCACTCGCCGTTACTAAGG | rDNA2.144[d] | | |

[a] *Drosophila yakuba* mtDNA map (Clary and Wolstenholme 1985).
[b] Sperling et al. 1994.
[c] K. Pruess, personal communication.
[d] T. Powers, personal communication.

**Table 4. Nei's genetic distance among 13 populations of stable fly**

| Population | SEW | HIC | VAL | PA1 | PA2 | PA3 | PA4 | GRI | STP | DAN | ELG | KER | BPTI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SEW | | 0.000 | 0.000 | 0.001 | 0.004 | 0.001 | 0.001 | 0.001 | 0.000 | 0.002 | 0.001 | 0.054 | 0.000 |
| HIC | | | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.055 | 0.000 |
| VAL | | | | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.046 | 0.000 |
| PA1 | | | | | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.051 | 0.000 |
| PA2 | | | | | — | 0.004 | 0.000 | 0.000 | 0.002 | 0.005 | 0.001 | 0.043 | 0.000 |
| PA3 | | | | | | — | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.062 | 0.000 |
| PA4 | | | | | | | | 0.000 | 0.000 | 0.002 | 0.000 | 0.053 | 0.000 |
| GRI | | | | | | | | | 0.000 | 0.001 | 0.000 | 0.043 | 0.000 |
| STP | | | | | | | | | | 0.000 | 0.000 | 0.057 | 0.000 |
| DAN | | | | | | | | | | | 0.001 | 0.060 | 0.001 |
| ELG | | | | | | | | | | | | 0.044 | 0.000 |
| KER | | | | | | | | | | | | | 0.067 |
| **BPT1** | | | | | | | | | | | | | |

calculating chi-square statistics with the following formulas $X^{2F}_{sT} = 2N\ (Fs_T)\ (k - 1)$ with $(k - 1)\ (s - I)$ df and $X^{2F}_{IT} = (FIT)^2 N(k - I)$ with k(k - I) df for k alleles and s populations.

Three levels of spatial variability were examined: among feedlots within regions (<40 km apart), between 2 regions separated by 130 km, and among spatially distant populations (>200 km apart). Sampling sites were grouped into the following 5 regions: (1) Lincoln, (2) Grand Island, (3) Elgin, NE, (4) Texas, and (5) Canada, based on their relative geographic distance from each other. All of the regions together formed the total population. Wright's *F* statistics were used to detect variation within and between Lincoln and Grand Island regions, samples within Nebraska relative to the Canadian sample, field samples relative to the laboratory sample, and variation among the temporal samples.

Tests for conformance to Hardy-Weinberg equilibrium (Swofford and Selander 1981) were conducted on 12 sample sites using a chi-square test with the Yates adjustment for small expected frequencies. Contingency chi-square tests, with Yates adjustment, were used on 4 polymorphic loci to test for homogeneity of allele frequencies.

## Results

**Enzyme Systems.** Of the 34 enzyme systems evaluated, 10 (ACOH, FDH, GCD, G3PDH, GAPDH, HADH, IDH, LDH, MDH, and PGDH) were used in this study (Table 2). These 10 enzyme systems provided 11 interpretable loci. Of the enzyme systems not used, 6 (ADH, ALPH, CAT, FBP, GO, and GDH) failed to stain, 8 (ACPH, FBA, DDH, FUMH, G6PDH, SOD, TRE, and XDH) were either too weak or inconsistent to score reliably, 3 (AAT, **MPI,** and PGI) could not be used because of blurry staining, and 7 (AO, EST, HK, CAP, MDHP, PGM, and TPI) had uninterpretable banding patterns.

Although HK exhibited a high degree of polymorphism for 2 apparent loci, it was not included in the analysis because of difficulty in interpreting the genetic basis of the phenotypes. Octanol dehydrogenase (ODH), stained the same enzyme as formaldehyde dehydrogenase (FDH). This phenomena was observed in blowflies (Taylor and Peterson 1994) and mammals (Hur and Edenberg 1992).

**Allozyme Phenotypes.** All systems used for this study had 1 locus, except for FDH which had 2, FDH-1 and FDH-2. *Acoh* and *Gcd* heterozygotes exhibited a 2-banded pattern characteristic of a monomeric structure. *Fdh-1, Hadh,* and *Pgdh* heterozygotes exhibited a 3-banded pattern characteristic of a dimeric structure. No heterozygotes for *G3pdh, Gapdh,* and *Mdh* were observed preventing characterization the structure of these isozymes.

**Population Structure.** Variation was detected in 7 of the 11 loci studied. Four loci (36%) were polymorphic (Table 5). An average of I.5 alleles per locus was observed. Three loci *(Gapdh, G3pdh,* and *Mdh)* were monomorphic, and 2 *(Gcd* and *Pgdh)* were polymorphic for all populations sampled with the exception of *Pgdh* in the PA3 collection. Two loci had rare alleles, $F < 0.01$ (Jones et al. 1991). One *Idh* (070/100) heterozygote was detected from Elgin, NE (ELG) and a *Fdh-2* (083/100) heterozygote was observed at St. Paul, NE (STP). Observed and expected levels of heterozygosity for field populations were 0.09 and 0.07, respectively (Table 6). Genotypic frequencies for 5 loci deviated significantly ($P < 0.05$) from Hardy-Weinberg equilibrium (PA1 *Gcd,* PA4 *Gcd,* GRI *Gcd, DAN Fdh-1,* and KER *Gcd).* Contingency chi-square analysis revealed no differentiation ($P < 0.05$) in allele frequencies among samples (Table 7).

Wright's *F* statistics (Table 7) revealed no departures from random mating. Chi-square test revealed no deviation ($P < 0.05$) from 0 for $Fs_T$ and $F_{IT}$ values (Table 8). Variation within populations $(F_{IS})$ from Nebraska and Canada was greater than variation between these populations $(Fs_T)$ (Table 8). Genetic differentiation was greatest between the field populations and the Kerrville laboratory population.

**Table 5. Gene frequencies for variable loci in 13 populations of stable fly**

| Locus | Allele° | | Population | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SEW | HIC | VAL | PA1 | PA2 | PA3 | **PA4** | GRI | STP | DAN | ELG | KER | BPT1 |
| *Acoh* | N | Mean | 75 | 75 | 50 | 75 | 49 | 75 | 46 | 49 | 49 | 50 | 50 | 74 | 49 |
| | 095 | 0.005 | 0.000 | 0.000 | 0.000 | 0.013 | 0.000 | 0.013 | 0.000 | 0.000 | 0.000 | 0.000 | 0.040 | 0.000 | 0.000 |
| | 100 | 0.972 | 1.000 | 0.967 | 0.980 | 0.947 | 1.000 | 0.987 | 1.000 | 0.980 | 0.969 | 1.000 | 0.920 | 0.912 | 0.969 |
| | 103 | 0.023 | 0.000 | 0.033 | 0.020 | 0.040 | 0.000 | 0.000 | 0.000 | 0.020 | 0.031 | 0.000 | 0.040 | 0.088 | 0.031 |
| | He | 0.054 | 0.000 | 0.060 | 0.040 | 0.100 | 0.000 | 0.030 | 0.000 | 0.040 | 0.060 | 0.000 | 0.150 | 0.160 | 0.060 |
| | $X^2$ | | 0.000 | 13.695° | 0.010 | 0.207 | 0.000 | 0.007 | 0.000 | 0.011 | 0.032 | 0.000 | 0.328 | 0.493 | 0.032 |
| *Fdh-1* | N | | 72 | 75 | 50 | 75 | 50 | 75 | 40 | 48 | 49 | 50 | 50 | 75 | 49 |
| | 094 | 0.001 | 0.000 | 0.000 | 0.010 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.010 |
| | 100 | 0.960 | 0.931 | 0.947 | 0.940 | 0.967 | 1.000 | 0.967 | 0.950 | 0.969 | 0.959 | 0.940 | 0.950 | 1.000 | 0.959 |
| | 114 | 0.039 | 0.069 | 0.053 | 0.050 | 0.033 | 0.000 | 0.033 | 0.050 | 0.031 | 0.041 | 0.060 | 0.050 | 0.000 | 0.031 |
| | He | 0.076 | 0.130 | 0.100 | 0.110 | 0.060 | 0.000 | 0.060 | 0.100 | 0.060 | 0.080 | 0.110 | 0.100 | 0.000 | 0.080 |
| | $\chi^2$ | | 0.359 | 0.207 | 0.168 | 0.424 | 0.000 | 0.710 | 0.082 | 0.033 | 0.066 | 25.628° | 0.110 | 0.000 | 0.066 |
| *Gcd* | N | | 69 | 45 | 48 | 71 | 44 | 34 | 50 | 45 | 49 | 50 | 48 | 74 | 48 |
| | 100 | 0.494 | 0.580 | 0.556 | 0.577 | 0.521 | 0.398 | 0.603 | 0.460 | 0.489 | 0.561 | 0.620 | 0.521 | 0.169 | 0.500 |
| | 121 | 0.496 | 0.420 | 0.444 | 0.423 | 0.479 | 0.602 | 0.397 | 0.540 | 0.511 | 0.439 | 0.380 | 0.479 | 0.831 | 0.500 |
| | He | 0.480 | 0.490 | 0.500 | 0.500 | 0.500 | 0.480 | 0.490 | 0.500 | 0.510 | 0.500 | 0.480 | 0.500 | 0.280 | 0.510 |
| | $\chi^2$ | | 0.001 | 1.135 | 0.181 | 6.013° | 0.279 | 0.029 | 35.440° | 6.800° | 1.796 | 0.438 | 2.803 | 6.137° | 2.228 |
| *Had-1* | N | | 75 | 75 | 50 | 75 | 50 | 75 | 50 | 49 | 49 | 50 | 50 | 74 | 49 |
| | A | 0.001 | 0.000 | 0.007 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | B | 0.991 | 0.973 | 0.987 | 1.000 | 0.993 | 0.970 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.990 | 1.000 | 0.980 |
| | C | 0.008 | 0.027 | 0.007 | 0.000 | 0.007 | 0.030 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.010 | 0.000 | 0.020 |
| | He | 0.016 | 0.052 | 0.030 | 0.000 | 0.010 | 0.060 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.020 | 0.000 | 0.040 |
| | $X^2$ | | 0.042 | 0.007 | 0.000 | 0.000 | 0.032 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.011 |
| Pgdh | N | | 69 | 74 | 50 | 75 | 50 | 75 | 40 | 48 | 49 | 49 | 50 | 74 | 49 |
| | 089 | 0.049 | 0.036 | 0.034 | 0.020 | 0.007 | 0.010 | 0.007 | 0.000 | 0.021 | 0.020 | 0.031 | 0.030 | 0.392 | 0.031 |
| | 100 | 0.893 | 0.841 | 0.899 | 0.920 | 0.927 | 0.939 | 0.959 | 0.920 | 0.948 | 0.918 | 0.918 | 0.910 | 0.608 | 0.908 |
| | 118 | 0.057 | 0.123 | 0.068 | 0.060 | 0.067 | 0.051 | 0.034 | 0.080 | 0.031 | 0.061 | 0.051 | 0.060 | 0.000 | 0.061 |
| | He | 0.175 | 0.270 | 0.180 | 0.150 | 0.140 | 0.120 | 0.080 | 0.150 | 0.100 | 0.150 | 0.110 | 0.170 | 0.480 | 0.170 |
| | $\chi^2$ | | 0.082 | 0.873 | 0.328 | 0.424 | 0.172 | 0.109 | 0.328 | 0.115 | 0.336 | 0.322 | 0.431 | 0.014 | 0.441 |

N, number of individuals in each sample; He, expected heterozygosity; $X^2$, deviation from the Hardy-Weinberg equilibrium.
° Populations not in equilibrium.

Nei's genetic distances (Nei 1978), D, among field collections averaged 0.001 (SEM = 0.001) and ranged from 0.000 to *0.005* (Table 4). Genetic distances among the 4 temporal collections from the Panama, NE, feedlot were <0.005. Genetic distance between field collections and KER colony ranged from 0.044 to *0.067.* Number of migrants *(Nm)* was calculated using the formula *([Nm* = (1 – *FST) /* 4FST]) (Wright 1951). The number of migrants needed to maintain the observed *FST (FST = 0.067)* value between the Canadian and Kerrville populations was 3.48 individuals per generation and *Nm* between the Lincoln and Grand Island localities was 11.8 individuals per generation *(FST* = 0.016).

**Polymerase Chain Reaction-Restriction Fragment Length Polymorphism.** DNA was extracted and amplified from frozen and alcohol preserved adults. Thirteen restriction sites were observed in amplicon I, 15 in amplicon II, *5* in amplicon III, and *9* in amplicon IV (Table *9).* The *46* restriction sites represent =I% of the mitochondria) genome. None of the restriction sites were polymorphic. Restriction analysis revealed 12 restriction sites within the 2-kb nuclear rDNA amplicon (Table *9)* and fragment patterns were identical for all sampled individuals.

**Table 6. Genetic variability in sampled stable fly populations**

| Population | % loci polymorphis° | Observed heterozygosity ±SE | Expected heterozygosity ±SE |
|---|---|---|---|
| SEW | 27.3 | 0.086 T 0.048 | 0.087 ± 0.048 |
| HIC | 27.3 | 0.087 ± 0.053 | 0.080 ± 0.046 |
| VAL | 27.3 | 0.078 ± 0.049 | 0.073 ± 0.045 |
| PA1 | 27.3 | 0.089 ± 0.058 | 0.075 ± 0.045 |
| PA2 | 18.2 | 0.064 ± 0.047 | 0.060 ± 0.044 |
| PA3 | 9.1 | 0.061 ± 0.045 | 0.060 ± 0.043 |
| PA4 | 27.3 | 0.107 ± 0.068 | 0.068 ± 0.046 |
| GRI | 18.2 | 0.047 ± 0.028 | 0.064 ± 0.045 |
| STP | 18.2 | 0.083 ± 0.053 | 0.074 ± 0.045 |
| DAN | 18.2 | 0.058 ± 0.047 | 0.061 ± 0.043 |
| ELC | 36.4 | 0.100 ± 0.056 | 0.087 ± 0.046 |
| KER | 33.3 | 0.086 ± 0.043 | 0.102 ± 0.048 |
| BPT1 | 18.2 | 0.094 ± 0.060 | 0.078 ± 0.046 |
| Mean[b] | 22.8 | 0.086 ± 0.019 | 0.070 ± 0.010 |

° Frequency of most common allele 0.95.
[b] Excludes KER population.

**Table 7. Summary of Wright (1978) F statistics for all stable fly collections**

| Locus | $F_{IS}$ | $F_{ST}$ | $F_{IT}$ |
|---|---|---|---|
| Acoh | 0.023 | 0.027 | 0.050 |
| Gcd | -0.154 | 0.051 | -0.095 |
| Had | -0.022 | 0.014 | -0.008 |
| Pgdh | -0.043 | 0.104 | 0.066 |
| Fdh-1 | 0.029 | 0.011 | 0.039 |
| Mean | -0.033 | 0.041 | 0.010 |

**Table 8. Nested analysis of variance of gene frequencies of stable fly populations**

| Contrast | Comparison | Variance component | $F_{xy}$ |
|---|---|---|---|
| Lincoln vs Grand Island region | Feedlot (region) | 0.006 | 0.008 |
| | Region (total) | -0.002 | -0.003 |
| | Feedlot (total) | 0.004 | 0.006 |
| Nebraska region vs Canada region | Feedlot (region) | 0.007 | 0.005 |
| | Region (total) | 0.001 | 0.001 |
| | Feedlot (total) | 0.008 | 0.006 |
| Field populations (NE and MB) vs laboratory (TX) population | Population (source) | 0.004 | 0.005 |
| | Source (total) | 0.035 | 0.041 |
| | Population (total) | 0.039 | 0.046 |

## Discussion

Geographical and temporal differentiation is low in the stable fly. This is characteristic of species that are a single, large panmictic population (Eanes and Koehn 1978). Genetic homogeneity among populations may be promoted by the dispersal capacity of the stable fly. Such dispersal will facilitate the spread of advantageous genes, such as insecticide resistance.

Wright's (1978) *FST* was greatest (0.041) comparing field samples to the Kerrville colony. This value was <0.05, indicating low levels of genetic differentiation of stable fly populations (Wright 1978). *FST* values among field populations essentially indicated no genetic differentiation. Low Nei's genetic distance values support the lack of differentiation between populations. The values of Nm indicated high levels of gene flow between populations (Schnabel and Hamrick 1990).

The percentage of polymorphic allozyme loci and mean heterozygosity in this study was intermediate to previous stable fly allozyme studies (Jones et al. 1991, Krafsur 1993). Mean heterozygosity (*He* = 0.096) and expected heterozygosity for *Acoh, Hadh, Fdh-I (Odh-2),* and *Pgdh* were similar between this study and Krafsur (1993). Although the lack of differentiation among populations in this study agreed with Jones et al. (1991), we observed higher levels of variability (*36%* polymorphic loci, *He* = 0.09) within populations. Five loci (Table 5) were the same as Jones et al. (1991), but variation for *Pgdh, Acoh,* and *Hadh* was greater in our study. We did not observe rare homozygotes as did Jones et al. (7991), although we did detect rare heterozygotes for *Fdh-2* and *Idh.* The average genetic distances (Nei 1978) between stable fly populations of 0.001 found in our study was comparable to that of Jones et al. (1991).

**Table 9. Estimated restriction fragment lengths for stable fly**

| Restriction enzyme | Amplicon | | | | |
|---|---|---|---|---|---|
| | I, 2.4 kb | H, 2.3 kb | HI, 1.5 kb | IV, 257 bp | ITS, 2.0 kb |
| *Abs* I | NU[a] | NU | NU | 133, 107 | NU |
| Apo I | NU | NU | NU | NU | 566, 446, 431, 378 |
| Ase I | 1460, 295, 225, 184, 176, 137 | NU | NU | 124, 88, 26 | 493, 461, 354, 342, 272 |
| Ava I | 1507, 1058 | NU | NU | 257 | 2005 |
| *Ban* II | 1774, 744, 300 | 1650, 650 | 1604 | 257 | 2005 |
| *Bfa* I | 2364 | 1100, 1000, 200 | 1604 | NU | NU |
| *Bsr* I | 2364 | 1415, 866 | 1253, 350 | NU | 1435, 516 |
| *Dde* I | 2364 | 2281 | 953, 386, 250 | 257 | NU |
| *Dpn* H | 2364 | 2281 | 754, 619, 231 | 257 | NU |
| *Dra* I | 1357, 825, 750 | 1150, 550, 450, 150 | 1604 | 257 | NU |
| *ECOR* I | 2364 | 1753, 527 | 1604 | 257 | 2005 |
| *EcoR V* | 1450, 1350 | 2150, 150 | 1604 | NU | 1107, 793 |
| *Hae* HI | 2364 | 2281 | 1604 | NU | NU |
| *Hine* H | 1937, 851 | 1450, 850 | 1604 | NU | 2005 |
| *Hind* HI | 2364 | 2281 | 1604 | NU | 1488, 517 |
| *Hpa I* | NU | NU | NU | NU | 2005 |
| *Hinf* I | 1029, 827, 412, 84 | 2281 | 1604 | 257 | NU |
| *Mse* I | NU | NU | NU | 81, 81, 30, 22, 14, 11 | NU |
| *Msp* I | 2364 | 2281 | 1604 | NU | 2005 |
| *Pvu* II | 2364 | 1150, 825, 325 | 1604 | NU | 2005 |
| *Rsa* I | 1087, 829, 750 | 2025, 275 | 1604 | NU | NU |
| Sau96I | NU | NU | NU | NU | 2005 |
| *ScrF* I | 2364 | 1416, 864 | 1604 | 257 | NU |
| Ssp I | NU | NU | NU | 128, 111 | NU |
| *aTaq* | NU | NU | NU | NU | 1206, 448, 348 |
| *Xba* I | NU | 1200, 1100 | NU | NU | NU |
| No. sites | 17 | 15 | 5 | 9 | 12 |

[a] NU, enzyme not used.

Restriction Fragment Length Polymorphism analysis of the nuclear and mitochondrial genomes revealed no variation within or among populations, supporting the allozyme evidence for minimal genetic differentiation. PCR–RFLP analyses on the same mtDNA amplicons used in this study have been conducted on 2 species of blow flies, primary screwworm, *Cochliomyia hominovorax* (Coquerel), and secondary screwworm, C. *rnacelleria (F.),* from North, Central, and South America (unpublished data). High levels of intraspecific variation were detected in primary screwworm, but like stable fly, the secondary screwworm exhibited a single haplotype. Levels of allozyme variation were similar for these 2 species (Taylor and Peterson 1994). The pattern of variation may be the result of the historical dispersal patterns (Avise 1987). Differences in allozyme and molecular variation illustrate the importance of using both as genetic markers (Milton 1994).

Nuclear rDNA provides an alternative to mtDNA for detecting genetic variation within and among populations, because of differences in the modes of inheritance (Weller et al. 1994). In this study, no detectable variation was observed in the rDNA gene family. This lack of variation in the nuclear genome is concordant with the lack of variation detected in the mitochondrial genome.

Several factors may contribute to low levels of genetic variability in stable fly populations. The stable fly probably was introduced to North American during the mid 18th century (Hall and Smith 1986). The colonizers likely consisted of individuals of European origin shipped with cattle across the Atlantic. Population bottlenecks are important during the establishment of new populations. Excessive contributions to the gene pool by a few individuals during this period (genetic drift) reduces the genetic variability in subsequent generations (founders effect). For example in the face fly, an estimated 50% of the alleles available in Europe were lost with its introduction to North America (Bryant et al. 1981). Finally, the period since the introduction of stable fly to North America is insufficient for the development of new variation. For the average animal mtDNA genome, 1 site substitution is expected every 3,000 years (Avise 1994).

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