

Population Genetics and Gene Variation of Stable Fly Populations (Diptera: Muscidae) in Nebraska

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J. Med. Entomol. 33(3): 413–420 (1996)

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 flow 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 (Skidmore 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, Krafus 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.

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-

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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 μ l 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 \approx 42% of the mitochondrial genome (Table 3). Amplicon I was 2.4 kb long and included portions of cytochrome oxidase (CO) I and II genes; amplicon II was 2.3 kb long and included portions of CO II and CO III genes; amplicon III was 1.5 kb long and included portions of CO III 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

Forward primer	Sequence 5'-3'	Reverse primer	Location ^a
Sp-1 ^b	TACAATTATCGCCTAAAGCTTAGCC	Sp-4 ^b	1435-1795
Control	GGTCAATGTTCAFAAATTTGTG	N3R2 ^c	5662-5942
	CCCTTGAATGTGGATTTGATC	N5F3 ^c	5725-7326
	GTAGGAGGAGCTGCTAATTC	N4F3 ^c	848-8737
	TTTCTACGGCTTACTAAGC	rDNA2	
		GAGACCATTACTGCTTTCAGTCA1CT	
		TCATTCATTCATGATATAGTCC	
		TGCTACTCTTAAGACAAATTAGG	
		GCCTATTCATTCGGTTGCTCA	
		GA7CCC7AGGTCACCTGCACATGCAT	

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

Popula- tion	SEW	HIC	VAL	PA1	PA2	PA3	PA4	GRI	STP	DAN	ELG	KER	BPT1
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 $\chi^2 F_{ST} = 2N (F_{ST}) (k - 1)$ with $(k - 1) (s - 1)$ df and $\chi^2 F_{IT} = (FIT)^2 N (k - 1)$ with $k(k - 1)$ 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 1.5 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 F_{ST} and F_{IT} values (Table 8). Variation within populations (F_{IS}) from Nebraska and Canada was greater than variation between these populations (F_{ST}) (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 ^a		Population												
			SEW	HIC	VAL	PA1	PA2	PA3	GRI	STP	DAN	ELG	KER	BPT1	
<i>Acoh</i>	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
<i>Fdh-1</i>	N	χ^2	0.000	13.695 ^a	0.010	0.207	0.000	0.007	0.000	0.011	0.032	0.000	0.328	0.493	0.032
	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
	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
<i>Gcd</i>	N	χ^2	0.359	0.207	0.168	0.424	0.000	0.710	0.082	0.033	0.066	25.628 ^a	0.110	0.000	0.066
	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
<i>Had-1</i>	N	χ^2	0.001	1.135	0.181	6.013 ^a	0.279	0.029	35.440 ^a	6.800 ^a	1.796	0.438	2.803	6.137 ^a	2.228
	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
	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
<i>Pgdh</i>	N	χ^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
	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
	He	0.175	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
			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
			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; χ^2 , deviation from the Hardy-Weinberg equilibrium.
^a 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 ($[Nm \cong (1 - FST) / 4FST]$) (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 $\approx 1\%$ of the mitochondrial 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 polymorphic ^a	Observed heterozygosity \pm SE	Expected heterozygosity \pm SE
SEW	27.3	0.086 \pm 0.048	0.087 \pm 0.048
HIC	27.3	0.087 \pm 0.053	0.080 \pm 0.046
VAL	27.3	0.078 \pm 0.049	0.073 \pm 0.045
PA1	27.3	0.089 \pm 0.058	0.075 \pm 0.045
PA2	18.2	0.064 \pm 0.047	0.060 \pm 0.044
PA3	9.1	0.061 \pm 0.045	0.060 \pm 0.043
PA4	27.3	0.107 \pm 0.068	0.068 \pm 0.046
GRI	18.2	0.047 \pm 0.028	0.064 \pm 0.045
STP	18.2	0.083 \pm 0.053	0.074 \pm 0.045
DAN	18.2	0.058 \pm 0.047	0.061 \pm 0.043
ELG	36.4	0.100 \pm 0.056	0.087 \pm 0.046
KER	33.3	0.086 \pm 0.043	0.102 \pm 0.048
BPT1	18.2	0.094 \pm 0.060	0.078 \pm 0.046
Mean ^b	22.8	0.086 \pm 0.019	0.070 \pm 0.010

^a 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	<i>F</i> _{IS}	<i>F</i> _{ST}	<i>F</i> _{IT}
			0.050
			-0.095
			-0.008
			0.066
			0.039
			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			
Nebraska region vs Canada region			
Field populations (NE and MB) vs laboratory (TX) population			

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, Krafur 1993). Mean heterozygosity (*He* = 0.096) and expected heterozygosity for *Acoh*, *Hadh*, *Fdh-1* (*Odh-2*), and *Pgdh* were similar between this study and Krafur (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. (1991), 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	II, 2.3 kb	IV, 257 bp	ITS, 2.0 kb
NU ^a	NU	NU	133, 107	NU
NU	NU	NU	NU	566, 446, 431, 378
1460, 295, 225, 184, 176, 137	NU	NU	124, 88, 26	493, 461, 354, 342, 272
1507, 1058	NU	NU	257	2005
1774, 744, 300	1650, 650	1604	257	2005
2364	1100, 1000, 200	1604	NU	NU
2364	1415, 866	1253, 350	NU	1435, 516
2364	2281	953, 386, 250	257	NU
2364	2281	754, 619, 231	257	NU
1357, 825, 750	1150, 550, 450, 150	1604	257	NU
2364	1753, 527	1604	257	2005
1450, 1350	2150, 150	1604	NU	1107, 793
2364	2281	1604	NU	NU
1937, 851	1450, 850	1604	NU	2005
2364	2281	1604	NU	1488, 517
NU	NU	NU	NU	2005
1029, 827, 412, 84	2281	1604	257	NU
NU	NU	NU	81, 81, 30, 22, 14, 11	NU
2364	2281	1604	NU	2005
2364	1150, 825, 325	1604	NU	2005
1087, 829, 750	2025, 275	1604	NU	NU
NU	NU	NU	NU	2005
2364	1416, 864	1604	257	NU
NU	NU	NU	128, 111	NU
NU	NU	NU	NU	1206, 448, 348
NU	1200, 1100	NU	NU	NU
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 hominivorax* (Coquerel), and secondary screwworm, *C. macelleria* (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 (Avisé 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 America 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 (Avisé 1994).

Acknowledgments

We thank Lloyd R. Criffield for assistance in collecting flies and Kenneth Pruess for assistance with primer selection. John Foster, James Petersen, and Steve Skoda provided helpful suggestions and critical reviews of the manuscript. This work was done in cooperation with the Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, and is published as Journal Series, Nebraska Agricultural Research Division paper 10721.

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Received for publication 5 July 1995; accepted 6 December 1995.