1995

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David B. Taylor
University of Nebraska-Lincoln, dave.taylor@ars.usda.gov

Richard D. Peterson II
University of Nebraska-Lincoln, padr20@yahoo.com

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DAVID B. TAYLOR AND RICHARD D. PETERSON II

Midwest Livestock Insects Research Laboratory, USDA–ARS, Department of Entomology, University of Nebraska, Lincoln, NE 68583

Ann. Entomol. Soc. Am. 88(5): 690–694 (1995)

ABSTRACT Allozyme variation in 4 populations of secondary screwworm, *Cochliomyia macellaria* (F.), representing North, Central, and South America was examined. Variability was observed in 12 of 13 enzyme loci, and the frequency of the most common allele was <0.95 for 6 loci. Observed and expected heterozygosities were 0.149 and 0.154, respectively. Nei's genetic distances were ≤0.001. Goodness-of-fit statistics for Hardy–Weinberg equilibrium and Wright's F_{IS} statistics indicated random mating within populations. Wright's F_{ST} statistics and chi-square contingency analyses indicated homogeneous gene frequencies among the populations. These data suggest that *C. macellaria* is a panmictic species with high levels of gene flow between populations.

KEY WORDS *Cochliomyia macellaria*, population genetics, isozymes

BLOW FLIES ARE among the earliest recognized insect plagues of mankind. Many species are associated with dermal and secondary traumatic myiasis, and a few species are agents of primary traumatic myiasis (Hall 1948). In addition to their propensity to infest wounds and "blow" meats, the attraction of blow flies to proteinaceous foods, including feces, garbage, fresh meats, and cheeses, has implicated them as vectors of filth-related diseases such as bacterial and amoebic dysentery, anthrax, and cholera (Hall 1948). Blow flies are also considered important indicators for time of death in forensic entomology (Greenberg 1991, Catts and Goff 1992). Despite their ubiquitous nature and economic importance, little is known concerning genetic variation within and between blow fly populations. Limited data exist on isozyme variation in the 2 species of primary screwworms, *Chrysomya bezziana* Villeneuve (Strong and Mahon 1991) and *Cochliomyia hominivorax* (Coquerel) (Taylor and Peterson 1994). However, other than Taylor and Peterson's (1994) study of *Cochliomyia macellaria* (F.) from northwestern Costa Rica, no information exists on the population structure of a saprophagous blow fly species.

Secondary screwworm, *Cochliomyia macellaria*, is a highly variable and widespread species. Thirty-two synonyms for this species have been published, including 9 by Robineau-Desvoidy alone (Dear 1985). *C. macellaria* is distributed throughout the New World from Canada to Argentina and the Caribbean (Dear 1985). *C. macellaria* is a sibling species to the primary New World screwworm, *C. hominivorax*. Although morphologically similar, *C. hominivorax* and *C. macellaria* are behaviorally very different. *C. hominivorax* is an obligate parasite of wounds of warm-blooded vertebrates, whereas *C. macellaria* is primarily saprophagous (Laake et al. 1936).

Our study was conducted in conjunction with studies of *C. hominivorax* population structure. We presume that the population structure of *C. macellaria* is representative of saprophagous blowflies and represents the ancestral state from which *C. hominivorax* was derived. By studying the similarities and differences between these 2 species, we hope to gain insight into the evolution and biology of parasitism in blow flies. The specific purpose of this study was to examine genetic variation in geographically distant populations of *C. macellaria*.

Materials and Methods

Specimens. Adult *C. macellaria* were collected from rotting liver, animal carcasses, or calves infested with *C. hominivorax*. Flies were collected from Lincoln, NE, in August 1993 (US), 3 sites in northwestern Costa Rica (CR) (Taylor and Peterson 1994), Tuxtla Gutierrez, Chiapas, Mexico, in August 1993 (MEXICO), and Seropedica, Rio de Janeiro, Brazil, in March 1994 (BRAZIL). Flies were stored in liquid nitrogen or at –80°C until used for the isozyme analysis.

Electrophoretic Techniques. Polyacrylamide gel electrophoresis was used for this study. Techniques were the same as those reported by Taylor and Peterson (1994). The head was removed from each fly and stored individually for use in future molecular genetic studies. Decapitated flies were ground in 150 μl of grinding buffer composed of

Table 1. Gene diversity at enzyme loci in *C. macellaria*

Enzyme	E.C. no.[a]	Symbol[b]	No. alleles	H_o[c]	H_e[d]
Aconitate hydratase	4.2.1.3	ACOH	6	0.187	0.200
Formaldehyde dehydrogenase	1.2.1.1	FDH	6	0.088	0.088
Fructose-biphosphate aldolase	4.1.2.13	ALD	1	0.000	0.000
Fumarate hydratase	4.2.1.2	FUMH	2	0.004	0.004
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH	2	0.002	0.002
Glycerol dehydrogenase	1.1.1.72	GCD	8	0.403	0.414
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	3	0.009	0.009
Hydroxyacid dehydrogenase	1.1.99.6	HADH	6	0.175	0.170
Isocitrate dehydrogenase	1.1.1.42	IDH	4	0.053	0.051
Malate dehydrogenase	1.1.1.37	MDH	4	0.015	0.015
Mannose-6-phosphate isomerase	5.3.1.8	MPI	10	0.614	0.645
Phosphogluconate 2-dehydrogenase	1.1.1.44	PGDH	6	0.192	0.207
Phosphoglucomutase	5.4.2.2	PGM	5	0.195	0.196
Mean ± SEM			5.08 ± 0.72	0.149 ± 0.051	0.154 ± 0.053

[a] Nomenclature Committee International Union of Biochemistry 1984.
[b] Murphy et al. (1990).
[c] Observed heterozygosity.
[d] Expected heterozygosity based upon Hardy-Weinberg equilibrium.

10% sucrose, 0.1% Triton X-100 (Sigma, St. Louis, MO), 0.37% EDTA, bromophenol blue and mercaptoethanol in 1:5 dilute Tris-citrate (TC) buffer (19 mM Trizma base [Sigma Chemical Co.], 5.6 mM citric acid, pH 7.1). Electrophoresis conditions were 6% polyacrylamide gels (14 by 16 cm and 1.5 mm thick) run at 300 V constant voltage for 3 h with Tris Borate EDTA (TBE) buffer (81 mM Trizma base, 28 mM boric acid, 14.6 mM EDTA, pH 8.9) or 4 h with TC buffer in vertical slab gel units (Hoeffer Scientific, San Francisco, CA). Initial currents were 0.027 (TBE) and 0.045 (TC) mA per gel, and ending currents were 0.015 (TBE) and 0.039 (TC) mA per gel. Buffer in the TC units was circulated between the upper and lower chambers to avoid overheating. Staining procedures, recipes, and allele nomenclature were the same as those used by Taylor and Peterson (1994). Thirteen enzyme systems were used for this study (Table 1).

Statistical Analysis. BIOSYS-1 (Swofford and Selander 1981) was used to compute population allele frequencies, expected and observed heterozygosities, test for fit with Hardy-Weinberg expectations, and calculate Nei's genetic distance (Nei 1978) and Wright's *F* statistics (Wright 1978). Significant deviation from zero of Wright's F_{ST} and F_{IT} statistics was determined by calculating chi-square statistics with the following formulas $\chi^2 F_{ST} = 2N(F_{ST})(k - 1)$ with $(k - 1)(s - 1)$ df and $\chi^2 F_{IT} = (F_{IT})^2 N(k - 1)$ with $k(k - 1)$ df for k alleles and s populations. Chi-square contingency analysis with a Yates adjustment for small expected frequencies was used to compare allele frequencies between populations. Flies from the 3 collections in Costa Rica were pooled for comparison with the other collections.

Results

Twelve of 13 isozyme loci were variable and 6 were polymorphic (frequency of most common allele <0.95) (Table 2). Expected and observed heterozygosities were 0.149 and 0.154, respectively. A mean of 5.08 alleles per locus was observed. Observed genotypes did not differ significantly from Hardy-Weinberg expectations (with pooling, see Swofford and Selander [1981], $P < 0.05$) except for *Pgd* in the US collection (excess homozygotes, $\chi^2 = 15.284$, df = 1, $P < 0.001$).

Wright's *F* statistics suggested little departure from random mating in *C. macellaria* populations. Among the individual locus F_{ST} and F_{IT} values, only F_{ST} for *Mpi* differed significantly from zero (Table 3). The mean F_{ST} value, 0.007, indicated that geographic differentiation accounts for <1% of the observed genetic variability. In fact, of the 65 alleles examined, frequencies among populations varied significantly only for the 112 allele of *Acoh* (Table 2). Overall allele frequencies did not differ among populations for any of the 13 loci studied and the total chi-square value, 94.287 (df = 159), was not significant. Nei's unbiased genetic distances were ≤0.001 (Table 4).

Discussion

The level of genetic variability observed in *C. macellaria* is similar to that observed in other cyclorrhaphous Diptera: *Musca autumnalis* De Geer (H_e = 0.18 [Krafsur and Black 1992]), *Musca domestica* L. (H_e = 0.11 [Black and Krafsur 1985]), *Haematobia irritans* (L.) (H_e = 0.14 [McDonald et al. 1987]), *Stomoxys calcitrans* (L.) (H_e = 0.09 [Krafsur 1993]), and *Cochliomyia hominivorax* (H_e = 0.14 [Taylor and Peterson 1994]). However, unexpectedly low levels of genetic differentiation among geographically distant populations were observed in *C. macellaria*. Comparable studies of insect species across such a broad geographic range are few. Munstermann (1980, 1984) reported genetic distances of 0.12–0.23 for populations of *Aedes epactius* Dyar and Knab distributed from Utah to El Salvador and 0.00–0.05 for *A. triseriatus*

Table 2. Allele frequencies for variable isozyme loci in *C. macellaria*

Locus	Allele	United States	Mexico	Costa Rica	Brazil	χ^{2a}
Acoh, n^b		100	50	282	98	
	100	0.005	0.010	0.002	0	0.185
	108	0.005	0	0.004	0	0.185
	112	0.085	0	0.044	0.031	10.336*
	116	0.065	0.060	0.053	0.056	0.192
	120	0.840	0.920	0.897	0.913	0.696
	124	0	0.010	0	0	2.129
	χ^{2c}	6.983	5.736	0.092	0.914	13.724[d]
Fdh, n^b		100	50	282	100	
	84	0.000	0.010	0.004	0.000	0.187
	100	0.020	0.030	0.020	0.010	0.051
	105	0.005	0	0.004	0	0.271
	114	0.955	0.920	0.952	0.980	0.730
	132	0.020	0.030	0.021	0.010	0.205
	148	0	0.010	0	0	2.128
	χ^{2c}	0.219	2.349	0.092	0.914	3.573[d]
Fumh, n^b		100	50	282	100	
	106	1.000	1.000	0.996	1.000	0.001
	153	0	0	0.004	0	0.452
	χ^{2c}	0.041	0.188	0.183	0.041	0.453[d]
Gapdh, n^b		100	50	282	100	
	100	1.000	1.000	0.998	1.000	0.000
	114	0	0	0.002	0	0.472
	χ^{2c}	0.188	0.094	0.002	0.188	0.472[d]
Gcd, n^b		100	47	282	97	
	36	0.025	0.053	0.035	0.036	0.772
	47	0.020	0	0.020	0.015	0.850
	53	0.005	0	0.009	0	0.864
	64	0.795	0.787	0.715	0.742	1.426
	77	0.005	0	0.014	0.010	0.841
	90	0.145	0.160	0.206	0.191	2.865
	100	0	0	0.002	0	0.465
	116	0.005	0	0	0.005	0.569
	χ^{2c}	3.140	2.161	2.771	0.578	8.651[d]
G3pdh, n^b		100	50	282	100	
	74	0.005	0.010	0.002	0.005	0.386
	100	0.990	0.990	0.998	0.995	0.004
	117	0.005	0	0	0	0.801
	χ^{2c}	0.601	0.135	0.185	0.270	1.191[d]
Hadh, n^b		100	50	282	99	
	90	0.005	0	0	0	0.800
	96	0.015	0.030	0.021	0	3.212
	108	0.885	0.940	0.904	0.934	0.298
	125	0.080	0.030	0.064	0.066	1.886
	130	0	0	0.002	0	0.470
	142	0.015	0	0.009	0	1.414
	χ^{2c}	2.038	2.042	0.425	3.572	8.077[d]
Idh, n^b		100	50	282	100	
	93	0.005	0	0	0	0.802
	100	0.965	0.990	0.966	0.995	0.118
	117	0.030	0.010	0.034	0	5.995
	129	0	0	0	0.005	0.802
	χ^{2c}	0.791	0.566	1.644	4.717	7.717[d]
Mdh, n^b		100	50	282	100	
	59	0	0	0.002	0	0.472
	92	1.000	1.000	0.988	0.995	0.015
	109	0	0	0.004	0.005	0.188
	114	0	0	0.005	0	0.704
	121	0	0	0.002	0	0.472
	χ^{2c}	0.396	0.526	0.538	0.391	1.850[d]
Mpi, n^b		100	50	282	97	
	94	0.005	0	0.004	0.010	0.386
	108	0.215	0.130	0.156	0.144	3.938
	112	0.010	0	0.032	0.021	4.026
	124	0.080	0.110	0.108	0.041	6.780
	130	0.005	0	0	0	0.787
	140	0.505	0.630	0.523	0.634	4.715
	155	0.010	0	0	0	3.868
	160	0.135	0.100	0.145	0.088	3.791
	180	0.005	0.010	0.012	0.010	0.232
	192	0.030	0.020	0.020	0.052	4.324
	χ^{2c}	4.980	3.545	6.453	13.214	28.192[d]
Pgm, n^b		100	50	282	100	
	85	0	0	0.002	0.010	1.734
	91	0.045	0.050	0.030	0.070	4.714
	94	0.020	0.010	0.011	0.015	0.433
	100	0.895	0.890	0.908	0.860	0.326
	108	0.040	0.050	0.048	0.035	0.317
	114	0	0	0.002	0.010	1.734
	χ^{2c}	0.314	0.382	2.011	6.551	9.258[d]
Pgdh, n^b		100	50	282	100	
	60	0.005	0	0	0	0.801
	100	0.020	0	0.016	0.010	0.825
	117	0.870	0.930	0.874	0.920	0.511
	133	0.015	0	0.012	0.010	0.405
	142	0.065	0.060	0.083	0.050	2.063
	167	0.005	0.010	0.012	0	1.516
	186	0.020	0	0.002	0.010	5.005
	χ^{2c}	4.447	1.316	3.040	2.326	11.128[d]

*, $P < 0.05$.

[a] Contingency chi-square values by allele (df = 3).

[b] Number of individuals examined from each population.

[c] Contingency chi-square values by population (df = no. alleles–1).

[d] Total contingency chi-square values by locus (df = [no. alleles–1] × 3).

(Say) populations from northern Michigan to southern Texas. Tabachnick and Powell (1979) reported a mean genetic distance of 0.0149 between New World populations of *Aedes aegypti* (L.). Bartlett et al. (1983) observed genetic distances of 0.017–0.508 among populations of boll weevil, *Anthonomus grandis grandis* Boheman, from the United States and Mexico. Steck (1991) found genetic distances between 0.05 and 0.137 between populations of the tephritid *Anastrepha fraterculus* (Wiedemann) from Brazil, Venezuela, Costa Rica,

Table 3. Wright (1978) *F* statistics for *C. macellaria* populations

Locus	F_{IS}	F_{ST}	F_{IT}
Acoh	0.073	0.010	0.082
Fdh	−0.031	0.006	−0.024
Fumh	−0.004	0.003	−0.001
Gapdh	0.002	0.001	0.000
Gcd	0.014	0.005	0.019
G3pdh	−0.008	0.002	−0.006
Hadh	0.039	0.006	−0.032
Idh	0.029	0.009	−0.019
Mdh	−0.007	0.004	−0.003
Mpi	0.013	0.010*	0.023
Pgm	0.005	0.003	0.008
Pgdh	0.087	0.006	0.092
Mean	0.018	0.007	0.025

*, Differs significantly from 0 ($P < 0.05$).

Table 4. Nei's unbiased genetic distances for *C. macellaria*

Location	1	2	3	4
1 United States	*****	0.001	0.000	0.001
2 Mexico		*****	0.001	0.000
3 Costa Rica			*****	0.001
4 Brazil				*****

and Mexico. In the only other species of blow fly for which isozyme data are available, Strong and Mahon (1991) obtained genetic distances as large as 0.098 between populations of *C. bezziana* from southern Africa, the Middle East, Malaysia, Indonesia, and Papua New Guinea. All of these values are an order of magnitude greater than the largest genetic distance, 0.001, observed between populations of *C. macellaria*.

Blow flies are strong-flying insects. *C. hominivorax* has been reported to fly as far as 290 km in 2 wk (Hightower et al. 1965), although normal lifetime dispersal is probably within the range of 3–25 km (Mayer and Atzeni 1993). The reliance of blow flies on patchy and ephemeral larval habitats requires females to disperse widely in search of oviposition sites. Because corpses are usually colonized by many females from diverse origins, the level of gene flow is potentially high. This dispersion and intermixing in each generation is probably responsible for the homogeneity of *C. macellaria* populations (Price 1980).

Acknowledgments

We thank Frank Parker and Robert Fischel for assistance in collecting in Costa Rica; owners and managers of Finca Bedilla, La Emilia, and Finca Montezuma for permission to collect on their properties; Gonzalo Moya-Borja, D. Marques Macedo, A. de Souza Leandro, and V. Pereira da Silva Junior for assistance in collecting in Brazil; and Troy Heller for technical assistance. John Foster, Shripat Kamble, and David Stanley-Samuelson provided helpful suggestions and critical reviews of the manuscript. This study was partially funded by Trust Fund Cooperative Agreement No. 58-5440-4-F009 provided by the Food and Agriculture Organization of the United Nations. 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 10877.

References Cited

Bartlett, A. C., W. C. Randall, and J. E. May. 1983. Allozyme variation among populations of boll weevils in Arizona and Mexico. Southwest. Entomol. 8: 118–130.

Black, W. C., IV, and E. S. Krafsur. 1985. Electrophoretic analysis of genetic variability in the house fly (*Musca domestica* L.). Biochem. Genet. 23: 193–203.

Catts, E. P., and M. L. Goff. 1992. Forensic entomology in criminal investigations. Annu. Rev. Entomol. 37: 253–272.

Dear, J. P. 1985. A revision of the New World Chrysomyini (Diptera: Calliphoridae). Rev. Bras. Zool. 3: 109–169.

Greenberg, B. 1991. Flies as forensic indicators. J. Med. Entomol. 28: 565–577.

Hall, D. G. 1948. The blowflies of North America. Thomas Say Foundation, vol. 4. Entomological Society of America, Lanham, MD.

Hightower, B. G., A. L. Adams, and D. A. Alley. 1965. Dispersal of released irradiated laboratory-reared screwworm flies. J. Econ. Entomol. 58: 373–374.

Krafsur, E. S. 1993. Allozyme variation in stable flies (Diptera: Muscidae). Biochem. Genet. 31: 231–240.

Krafsur, E. S., and W. C. Black IV. 1992. Analysis of isozyme loci in the face fly, *Musca autumnalis* DeGeer. Biochem. Genet. 30: 625–634.

Laake, E. W., E. C. Cushing, and H. E. Parish. 1936. Biology of the primary screwworm fly, *Cochliomyia americana*, and a comparison of its stages with those of *C. macellaria*. U.S. Dep. Agric. Tech. Bull. 500.

Mayer, D. G., and M. G. Atzeni. 1993. Estimation of dispersal distances for *Cochliomyia hominivorax* (Diptera: Calliphoridae). Environ. Entomol. 22: 368–374.

McDonald, P. T., L. R. Hilburn, and S. E. Kunz. 1987. Genetic similarities among natural populations of the horn fly (Diptera: Muscidae). Ann. Entomol. Soc. Am. 80: 288–292.

Munstermann, L. E. 1980. Distinguishing geographic strains of the *Aedes atropalpus* group (Diptera: Culicidae) by analysis of enzyme variation. Ann. Entomol. Soc. Am. 73: 699–704.

1984. Geographic patterns of genetic variation in the treehole mosquito *Aedes triseriatus*, pp. 327–343. *In* L. P. Lounibos, J. R. Rey, and J. H. Frank [eds.], Ecology of mosquitoes. Florida Medical Entomology Laboratory, Vero Beach.

Murphy, R. W., J. W. Sites, Jr., D. G. Buth, and C. H. Haufler. 1990. Proteins I: isozyme electrophoresis, pp. 45–126. *In* D. M. Hillis and C. Moritz [eds.], Molecular systematics. Sinauer, Sunderland, MA.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Nomenclature Committee International Union of Biochemistry. 1984. Enzyme nomenclature. Academic, New York.

Price, P. W. 1980. Evolutionary biology of parasites. Princeton University Press, Princeton, NJ.

Steck, G. J. 1991. Biochemical systematics and population genetic structure of *Anastrepha fraterculus* and related species (Diptera: Tephritidae). Ann. Entomol Soc. Am. 84: 10–28.

Strong, K. L., and R. J. Mahon. 1991. Genetic variation in the Old World screwworm fly, *Chrysomya bezziana* (Diptera: Calliphoridae). Bull. Entomol. Res. 81: 491–496.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics, release 1.7. J. Hered. 72: 281–283.

Tabachnick, W. J., and J. R. Powell. 1979. A worldwide survey of genetic variation in the yellow fever mosquito, *Aedes aegypti*. Genet. Res. 34: 215–229.

Taylor, D. B., and R. D. Peterson II. 1994. Population genetics and gene variation in primary and secondary screwworm (Diptera: Calliphoridae). Ann. Entomol. Soc. Am. 87: 626–633.

Wright, S. 1978. Evolution and the genetics of populations, vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.

Received for publication 26 October 1994; accepted 6 April 1995.