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Author(s): Thomas A. Gavin, Ronald A. Howard and Bernie May

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ALLOZYME VARIATION AMONG BREEDING POPULATIONS OF RED-WINGED BLACKBIRDS: THE CALIFORNIA CONUNDRUM

THOMAS A. GAVIN, RONALD A. HOWARD,¹ AND BERNIE MAY

Department of Natural Resources, Cornell University, Ithaca, New York 14853 USA

ABSTRACT.—We examined allozyme variation in 10 breeding populations of Red-winged Blackbirds (*Agelaius phoeniceus*) across the United States, which represented samples from 9 of 14 putative subspecies in North America. Variation at 13 of 28 resolvable loci revealed a high level of genetic similarity for all seven populations from Florida and New York through the Great Plains to Oregon and northeastern California (pairwise Nei's distances, all ≤ 0.004). Differences in allozyme frequencies we found suggest that fewer subspecies exist in the continental United States than are currently recognized. The most interesting result was that the genetic distance between the populations sampled at Sacramento and San Francisco Bay national wildlife refuges, which are only 214 km apart, had a Nei's distance approximately 10 times as great as the genetic distance between Florida and Oregon populations. Salton Sea, California, the remaining population sampled, was also highly differentiated. Strong site fidelity, the nonmigratory behavior of populations at Salton Sea and San Francisco, or both probably explain their relative allozyme distinctness, but the possibility that the brackish environment in which these birds live enforces a selective regime that reduces successful immigration or emigration to other habitats is intriguing. Received 20 August 1990, accepted 10 January 1991.

GENETIC variation present in bird populations is a reflection of each population's evolutionary history and a potential tool for investigating that population's relationship to other conspecific populations. Understanding the genetic structure of avian populations has lagged behind similar work on mammals, in part because only recently have those interested in genetic structure of natural populations believed there was enough variation in birds for use in quantifying similarities and differences among populations (Barrowclough and Johnson 1988). In addition, to understand population structure in migratory birds, samples must be obtained at a particular time of year from relatively discrete geographic areas that represent breeding populations or demes. The genetic structure of populations cannot be determined by sampling conspecific individuals during migration, although a crude estimate of genetic variation present in the species could be assayed in this way (e.g. Barrowclough and Corbin 1978).

The recent work of Zink (1986) represents an illustrative example of the approach that attempts to characterize relationships among con-

specific breeding populations using allozyme data. It is apparent that no one method of analyzing this type of data can provide a complete answer to the amount of present or historical gene flow between populations (Slatkin 1985), but that a diversity of analytical techniques should be employed to develop the most thorough description possible before conclusions are drawn.

Red-winged Blackbirds (*Agelaius phoeniceus*) are one of the most numerous and widespread passerines in North America, with breeding populations from the east coast to the west coast and from Alaska to Costa Rica (AOU 1983). *Agelaius phoeniceus* is subdivided into 14 subspecies in North America (AOU 1957), which are distinguished by length of wings and tails, body size, shape and size of bill, and coloration of the plumage (Van Rossem 1926, Power 1970). An additional 12 subspecies occur in Central America and the Caribbean (Howard and Moore 1980). Northern populations tend to be migratory, while southern populations are not. Red-winged Blackbirds exhibit breeding-site fidelity (e.g. Nero 1956a, b; Howard 1977; Beletsky and Orians 1987), but similar to most birds, Red-winged Blackbirds are capable of long distance movement. Distances between breeding and wintering areas can approach 1,125 km (Dolbeer 1978).

¹ Present address: State 4-H Office, 809 University Drive East, Texas A&M University, College Station, Texas 77843-2473 USA.

TABLE 1. Sampling locations, sample size, migratory status, age of individuals sampled, and subspecies for 10 breeding populations (9 subspecies) of Red-winged Blackbirds (*Agelaius phoeniceus*).

Location (acronym)	n	Status ^a	Age	Subspecies
Merritt Island NWR, Brevard Co., FL (FL-MI)	40	1	Adults	<i>mearnsi</i>
San Francisco Bay NWR, San Mateo and Alameda cos., CA (CA-SF)	35	2	Nest.	<i>mailliardorum</i>
Sacramento NWR, Glenn Co., CA (CA-SAC)	40	3	Adults	<i>californicus</i>
Monte Vista NWR, Rio Grande Co., CO (CO-MV)	31	4	Nest.	<i>fortis</i>
Salton Sea NWR, Imperial Co., CA (CA-SALT)	20	1	Adults	<i>sonoriensis</i>
J. Clark Salyer NWR, McHenry Co., ND (ND-SAL)	24	4	Nest.	<i>arctolegus</i>
Cornell Biol. Field Station, Madison Co., NY (NY-COR)	23	4	Nest.	<i>phoeniceus</i>
Turnbull NWR, Spokane Co., WA (WA-TURN)	11	4	Nest.	<i>nevadensis</i>
Klamath Wildl. Manage. Area, Klamath Co., OR (OR-KLA)	43	4?	Adults	<i>caurinus</i>
Modoc NWR, Modoc Co., CA (CA-MOD)	25	4?	Adults	<i>nevadensis</i>

^a 1 = largest population occurs in winter because of birds that have moved onto refuge; migratory behavior of birds that breed here is unknown. 2 = abundant year round. 3 = most birds are gone in winter. 4 = definitely migratory; no birds present in winter.

In contrast to the vast literature on the ecology and behavior of Red-winged Blackbirds, almost no work has focused on the use of biochemical techniques to quantify genetic variation or to examine population differentiation. Polymorphisms in egg-white protein from Washington (Brush 1970) and the Northeast (Spendelov 1980) have been described, but these studies examined a small number of loci over a limited geographic area. Cox and James (1984) found no karyotypic invariants from Red-winged Blackbirds sampled in Colorado, Minnesota, and Florida. Finally, Ball et al. (1988) sampled Red-winged Blackbirds from 14 states, Canada, and Mexico, and found little population differentiation in mtDNA. They concluded that historically high levels of gene flow resulted in the low observed phylogeographic differentiation.

The objectives of this study were to provide an extensive electrophoretic survey of allozyme variation in breeding populations of Red-winged Blackbirds, to examine whether geographic distance among breeding populations sampled was related to genetic distance, and to relate these observations to the present subdivision of *A. phoeniceus* into subspecies.

METHODS

Requests for samples were sent to 33 national wildlife refuges (NWR) in 16 states. Samples were obtained from the Cornell Biological Field Station, Madison County, New York, and 7 refuges: Merritt Island NWR, Brevard County, Florida; San Francisco Bay NWR, San Mateo and Alameda counties, California; Sacramento NWR, Glenn County, California; Monte Vista NWR, Rio Grande County, Colorado; Salton Sea

NWR, Imperial County, California; J. Clark Salyer NWR, McHenry County, North Dakota; Turnbull NWR, Spokane County, Washington. Refuge personnel were asked to collect Red-winged Blackbirds by either randomly sampling one nestling from each of ≥ 20 nests or shooting ≥ 20 territorial males. All individuals were collected at each site during a 1–2 day period in spring 1985, packed in dry ice, and shipped by overnight carrier to our laboratory. Additional samples were obtained in 1989 from Modoc NWR, Modoc County, California, and Klamath Wildlife Management Area, Klamath County, Oregon. We obtained a total of 292 Red-winged Blackbirds (Table 1). Each population was located within the geographic range of a different subspecies, except that two populations were sampled within the range of *A. p. nevadensis* (Fig. 1).

A composite tissue sample of approximately equal-sized portions of breast muscle, liver, and heart was taken from each bird. An equivalent volume of 0.05 M Tris HCl (pH 7.1) was added to each sample, and extraction was performed with a 8-mm glass rod in 12 x 75 mm disposable test tubes. After centrifugation the extracts were subjected to horizontal starch gel electrophoresis (May et al. 1979; Sigma and Connaught starch mixed 1:1 by weight) coupled with histochemical staining protocols (Harris and Hopkinson 1976). We examined 28 genetic loci for variation in a preliminary sample of 40 birds (5 from each of the 8 populations sampled in 1985).

Presumptive genotypes were assigned to each individual based on the enzyme banding patterns observed. To analyze genotypic data, we used the computer program GENES IN POPULATIONS (designed by B. May and C. C. Krueger, and written in C by W. Eng), which tests each population for Hardy-Weinberg equilibrium, performs the pool-clustering algorithm (Royse and May 1982, May and Krueger 1990), and calculates Nei's (1972) genetic distance (*D*), Wright's *F*-statistics (Nei 1973), and heterozygosity. We used *G*-statistics to test for differences in allele

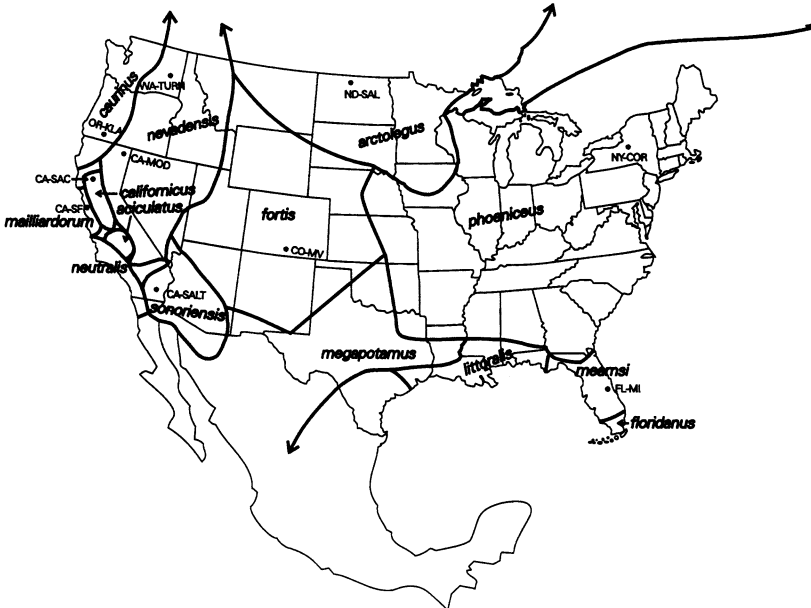


Fig. 1. Map showing the putative subspecies of Red-winged Blackbirds in the continental U.S. and the 10 sampling locations for this study. See Table 1 for explanation of acronyms.

frequencies among populations. A probability level of 0.01 rather than 0.05 was used to conclude statistical significance, because of the large number of pairwise comparisons we examined.

We used Slatkin's method to estimate gene flow among populations (Barton and Slatkin 1986; Slatkin 1981, 1985; Slatkin and Barton 1989). Slatkin's methods use information on the conditional average frequency ($\bar{p}(i)$) of all alleles found in the populations of interest, and on "private" alleles found only in one population other than the population being held out of analysis in any particular iteration. These two methods produce curves that illustrate whether gene flow is low, medium, or high relative to other organisms, and an estimate of Nm . The product of N , the effective population size, and m , the immigration rate, represents an estimate of the number of immigrants per generation.

RESULTS

GENETIC VARIATION WITHIN BLACKBIRD POPULATIONS

Of 28 loci resolved in the preliminary survey of 40 individuals, 13 loci were polymorphic (Table 2). All remaining individuals were examined only at these 13 loci and were assumed to be homozygous at the 15 monomorphic loci for the same allele found in the first 40 individuals. The 15 monomorphic loci were Aat-1, Aat-2

(2.6.1.1), Ak-1 (1.7.4.3), Gpt (2.6.1.2), Ldh-1, Ldh-2 (1.1.1.27), α -Man (3.2.1.24), Mdh-1, Mdh-2 (1.1.1.37), Mup (3.1.3.2), Pep-Pap-2 (3.4.-.-), Pro-5, Sod-1, Sod-2 (1.15.1.1), and Tpi (5.3.1.1). The number of polymorphic loci per population (see Table 1 for explanation of acronyms) ranged from 6–9 (21–32%; Table 2). Average heterozygosity (H_{obs}) across all populations was 0.037 (± 0.014), and ranged from 0.019 (± 0.010) at FL-MI to 0.055 (± 0.023) at CA-SALT. Expected heterozygosity (H_{exp}) was 0.038 (± 0.013). Allelic frequencies were not in Hardy-Weinberg equilibrium at only one locus in one population (out of a possible 79 tests); the Gda locus at WA-TURN exhibited a deficiency of heterozygotes ($P < 0.025$). The average number of alleles per locus for each population, averaged for all 28 loci, ranged from 1.25 (OR-KLA) to 1.57 (ND-SAL) (Table 2).

GENETIC VARIATION AMONG POPULATIONS

Genetic distance.—The largest Nei's distance (based on all 28 loci) observed was 0.031 between CA-SF and CA-MOD (Table 3). No population was fixed for an allele not found in another population, although major differences observed among populations occurred at the Gr locus. Allele 100 was the most common allele

TABLE 2. Allelic frequencies at 13 polymorphic loci in 10 breeding populations of Red-winged Blackbirds (*Agelaius phoeniceus*). * = $P < 0.01$.

Locus (EC number)	F_{ST}	Allele	Populations									
			FL- MI	CA- SF	CA- SAC	CO- MV	CA- SALT	ND- SAL	NY- COR	WA- TURN	OR- KLA	CA- MOD
Ck-1 (2.7.3.2)	0.043	-100	1.000	1.000	0.975	1.000	0.925	1.000	1.000	0.955	1.000	1.000
		-140	0.000	0.000	0.025	0.000	0.075	0.000	0.000	0.000	0.045	0.000
Ck-2 (2.7.3.2)	0.055	100	1.000	1.000	1.000	1.000	0.925	1.000	0.978	1.000	1.000	1.000
		120	0.000	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.000
		116	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000
G3p (1.1.1.8)	0.144*	-100	1.000	1.000	1.000	0.984	0.800	0.979	1.000	1.000	0.988	1.000
		-79	0.000	0.000	0.000	0.016	0.200	0.021	0.000	0.000	0.000	0.000
		-111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000
Gda (3.5.4.3)	0.076*	100	1.000	0.971	1.000	0.871	1.000	0.958	1.000	0.818	0.953	0.958
		96	0.000	0.029	0.000	0.129	0.000	0.042	0.000	0.182	0.047	0.042
Gpi (5.3.1.9)	0.020	-100	1.000	0.971	0.963	1.000	0.975	1.000	1.000	0.955	1.000	0.980
		0	0.000	0.014	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.020
		100	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.045	0.000	0.000
		-29	0.000	0.014	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gr (1.6.4.2)	0.399*	100	0.885	0.100	0.625	0.758	0.350	0.896	0.913	0.864	0.953	1.000
		162	0.026	0.843	0.325	0.016	0.625	0.000	0.000	0.000	0.047	0.000
		62	0.090	0.057	0.050	0.226	0.025	0.104	0.087	0.091	0.000	0.000
		235	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000
Idh-1 (1.1.1.42)	0.015	-100	1.000	1.000	0.988	1.000	1.000	0.979	1.000	1.000	1.000	0.980
		-121	0.000	0.000	0.013	0.000	0.000	0.021	0.000	0.000	0.000	0.000
		-111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020
Idh-2 (1.1.1.42)	0.049*	-100	0.975	0.886	0.913	1.000	0.975	1.000	1.000	1.000	0.988	0.940
		0	0.025	0.114	0.087	0.000	0.025	0.000	0.000	0.000	0.012	0.060
Mpi (5.3.1.8)	0.026	100	0.975	0.971	1.000	0.984	1.000	0.917	0.978	0.955	0.942	0.980
		95	0.000	0.000	0.000	0.016	0.000	0.000	0.022	0.000	0.000	0.020
		90	0.013	0.000	0.000	0.000	0.000	0.021	0.000	0.045	0.000	0.000
		83	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Np (2.4.2.1)	0.014	100	0.988	0.957	0.975	0.968	0.975	0.938	0.978	1.000	1.000	1.000
		60	0.000	0.014	0.013	0.000	0.000	0.021	0.022	0.000	0.000	0.000
		115	0.013	0.014	0.000	0.016	0.025	0.021	0.000	0.000	0.000	0.000
		130	0.000	0.014	0.013	0.016	0.000	0.021	0.000	0.000	0.000	0.000
Pepgl-1 (3.4.—.—)	0.029	100	0.925	0.929	0.813	0.903	0.900	0.938	0.913	0.773	0.814	0.820
		111	0.063	0.071	0.175	0.097	0.100	0.042	0.022	0.136	0.151	0.160
		87	0.013	0.000	0.013	0.000	0.000	0.021	0.043	0.091	0.035	0.020
		124	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000
Pepgl-2 (3.4.—.—)	0.071*	100	0.988	0.771	0.850	0.790	0.875	0.958	0.870	0.909	1.000	0.980
		107	0.000	0.229	0.150	0.210	0.100	0.021	0.043	0.091	0.000	0.020
		90	0.013	0.000	0.000	0.000	0.025	0.021	0.022	0.000	0.000	0.000
		83	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000	0.000
Pgd (1.1.1.44)	0.032	100	1.000	0.986	0.988	0.984	1.000	0.935	0.957	0.955	1.000	0.900
		124	0.000	0.000	0.000	0.000	0.000	0.022	0.043	0.000	0.000	0.040
		164	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.045	0.000	0.000
		53	0.000	0.014	0.013	0.000	0.000	0.022	0.000	0.000	0.000	0.060
		86	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000
H _{ob}			0.019	0.042	0.054	0.047	0.055	0.034	0.023	0.045	0.026	0.027
SE			0.010	0.017	0.023	0.022	0.023	0.011	0.011	0.020	0.014	0.013
H _{exp}			0.018	0.044	0.052	0.045	0.058	0.034	0.028	0.051	0.023	0.030
SE			0.009	0.017	0.022	0.019	0.022	0.011	0.011	0.019	0.012	0.013
# polymorphic loci			6	9	9	8	9	9	7	8	6	8
Mean # alleles per locus			1.32	1.46	1.46	1.36	1.39	1.57	1.39	1.36	1.25	1.32

at the Gr locus in every population (fixed in CA-MOD), except at CA-SF and CA-SALT, where it occurred at frequencies of 0.100 and 0.350, respectively (Table 2).

All six Red-winged Blackbird populations outside California and CA-MOD, which represented six putative subspecies, were similar

in allele frequencies (Table 2, Fig. 2). Allele frequencies were not significantly different among FL-MI, NY-COR, and ND-SAL or between OR-KLA and CA-MOD at the 0.01 level. In fact, Nei's distance between ND-SAL and FL-MI was 0.000. A combined pool of allele frequencies of the first three populations was sig-

TABLE 3. Nei's genetic distances (\bar{D}) (above diagonal) and P -value of G -test for genotypic differentiation and F_{ST} values (below diagonal) for pairwise combinations of Red-winged Blackbird (*Agelaius phoeniceus*) populations. See Table 1 for explanation of acronyms.

	FL-MI	CA-SF	CA-SAC	CO-MV	CA-SALT	ND-SAL	NY-COR	WA-TURN	CA-MOD	OR-KLA	\bar{D}^a	\bar{F}_{ST}^b
FL-MI	—	0.026	0.004	0.003	0.014	0.000	0.001	0.002	0.001	0.001	0.006	0.071
CA-SF	<0.005 0.288	—	0.011	0.023	0.005	0.028	0.027	0.028	0.031	0.028	0.023	0.214
CA-SAC	<0.005 0.058	<0.005 0.099	—	0.004	0.006	0.005	0.005	0.005	0.006	0.005	0.006	0.058
CO-MV	<0.005 0.043	<0.005 0.193	<0.005 0.039	—	0.014	0.002	0.002	0.002	0.004	0.004	0.006	0.063
CA-SALT	<0.005 0.155	<0.005 0.044	<0.005 0.045	<0.005 0.111	—	0.016	0.016	0.016	0.018	0.016	0.013	0.121
ND-SAL	>0.1 0.008	<0.005 0.251	<0.005 0.056	<0.01 0.029	<0.005 0.138	—	0.001	0.002	0.001	0.001	0.006	0.062
NY-COR	<0.05 0.013	<0.005 0.266	<0.005 0.057	<0.005 0.031	<0.005 0.148	>0.1 0.010	—	0.002	0.002	0.001	0.006	0.067
WA-TURN	<0.005 0.034	<0.005 0.215	<0.005 0.046	>0.1 0.020	<0.005 0.123	>0.1 0.024	<0.05 0.029	—	0.002	0.002	0.007	0.060
CA-MOD	<0.01 0.025	<0.005 0.285	<0.005 0.062	<0.005 0.052	<0.005 0.164	<0.05 0.021	<0.025 0.025	<0.025 0.025	—	0.001	0.007	0.074
OR-KLA	<0.025 0.018	<0.005 0.287	<0.005 0.059	<0.005 0.053	<0.005 0.157	<0.01 0.019	<0.005 0.027	<0.005 0.023	<0.05 0.011	—	0.007	0.073

^a Mean Nei's distances for each population listed along left of table.

^b Mean F_{ST} values for each population listed along left of table.

nificantly different from a pool of the latter two, but when these five populations were pooled and compared against WA-TURN, the next population added in the phenogram (Fig. 2), they were not significantly different. That is, allele frequencies of every population or cluster of populations in the phenogram to the right of node A was significantly different from the pool of populations already added. Nei's distance, however, was only 0.004 at the node where CA-SAC joined the seven populations described above (Fig. 2).

The most interesting result, based on pool clustering of genetic distance coefficients, was that CA-SAC clustered more closely with the five populations outside California plus CA-MOD than with either of the other California populations, even though CA-SF was the population nearest CA-SAC geographically. For example, the genetic distance between FL-MI and OR-KLA ($D = 0.001$) was less (approx. 1/10) than the genetic distance between CA-SAC and CA-SF ($D = 0.011$) (Table 3). CA-MOD and OR-KLA had nearly identical Nei's distance with respect to their distance from each of the other California populations. The D between CA-MOD and WA-TURN, the only two populations of the same putative subspecies, was 0.002, and was as great or greater than Nei's distance be-

tween CA-MOD and four other populations, all of which are thought to be different subspecies.

This arrangement is obviously not congruent with physical distance among these populations. The CA-SF and CA-SAC populations are only 219 km apart, yet CA-SAC and FL-MI, which have a smaller genetic distance between them, are 3,900 km apart. A regression of pairwise Nei's distances with a corresponding matrix of pairwise geographic distances (km) among all sampling locations (Fig. 3) produced no statistically significant relationship between these two measures ($r^2 = 0.008$).

F_{ST} values.—The overall F_{ST} when all 10 populations were included was 0.165. That is, nearly 17% of all the allozyme variation that we quantified was accounted for by among-population variability, which represents significant population structuring in an avian species (see Barrowclough and Johnson 1988: table 1; Evans 1987: table 2). If the four California populations were omitted, the F_{ST} among the remaining six populations dropped to 0.043. Pairwise F_{ST} values among all possible combinations (45) of two populations ranged from 0.008 (FL-MI and ND-SAL) to 0.288 (FL-MI and CA-SF) (Table 3). Average F_{ST} values were less than 0.075 for every population except CA-SF (0.214) and CA-SALT (0.121) (Table 3). Nei's distances and F_{ST} values

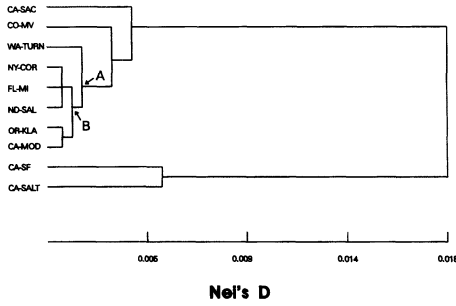


Fig. 2. A phenogram of genetic distances (Nei 1972) using the pool clustering algorithm of Royse and May (1982) based on 28 loci in 10 breeding populations of Red-winged Blackbirds.

between pairs of populations were highly correlated ($r^2 = 0.972$, Fig. 4), as one might expect given the reliance of each measure on the same data.

Analysis of rare alleles.—The analysis of private alleles indicated a trend ($Nm = 5.1$) of high gene flow among nine populations. The Washington population was not included in any analyses of rare alleles because only 11 individuals were sampled from this location. When $Nm > 1$, gene flow is thought to be substantial (Slatkin 1985). This type of analysis provides additional insights into the contribution of individual demes to the overall pattern. The lowest Nm value (4.3) occurred when CA-MOD was held out of the analysis, which indicates this population contributed to the higher estimate of Nm (i.e. >5) when it was included. The highest Nm value (6.9) occurred when CA-SALT was

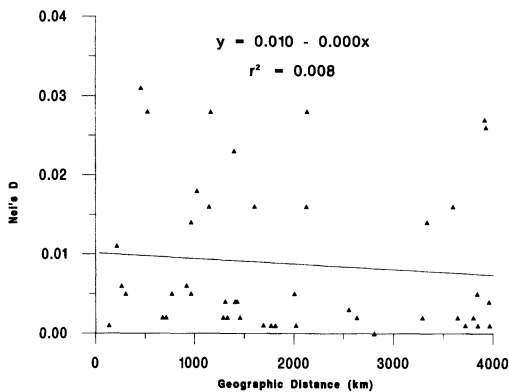


Fig. 3. Scatter diagram of Nei's genetic distance against straight-line geographic distance for each of 45 pairwise comparisons between sampling locations.

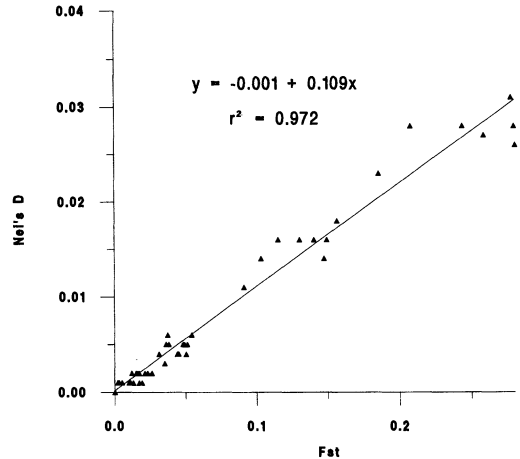


Fig. 4. Scatter diagram of Nei's genetic distance against F_{ST} values for each of 45 pairwise comparisons between sampling locations.

held out of analysis, which suggests that gene flow to and from CA-SALT relative to all other demes was the lowest.

DISCUSSION

Morphological differences exist among these populations (e.g. Van Rossem 1926). There are enough differences to describe nine subspecies in the 10 breeding populations we sampled. The experiments of James (1983), however, suggest these differences may not be genetic. Red-winged Blackbird eggs were transplanted between nests in southern and northern Florida and from Colorado to Minnesota (both transplants crossed boundaries of putative subspecies), and the morphology of the transplanted, foster young relative to controls from their home site was monitored. The somewhat surprising results were that ratios of certain morphological characters of the transplanted nestlings shifted in the direction of their foster parents relative to the controls (James 1983).

Our results are consistent with the general conclusion that gene flow is (or was historically) relatively high among the 10 populations we sampled. The plot of conditional average frequencies of alleles against the proportion of demes with these alleles is quite similar to avian species thought to have high levels of gene flow (Slatkin 1981), and our curve (not shown) is nearly identical in form to that for Northern Flicker (*Colaptes auratus*; Grudzien et al. 1987),

for White-crowned Sparrow (*Zonotrichia leucophrys*; Corbin and Wilkie 1988), for Western Flycatcher (*Empidonax difficilis*; Johnson and Marten 1988), and Cory's Shearwater (*Calonectris diomedea*; Randi et al. 1989). However, genetic distances between Red-winged Blackbird populations are not concordant with geographic distances among these same populations.

Certainly the genetic distances among the Florida, New York, and North Dakota populations (≤ 0.001) were so small as to place doubt on any subspecific differentiation across this region; the pairwise comparisons of allele frequencies among these three populations were not statistically significant (two had $P > 0.10$, and one had $P > 0.01$). This finding is important, because our experience indicates that allele frequencies between any two noninterbreeding populations are almost always significantly different statistically, even when genetic distances are very small. After these three populations are joined with the Oregon and California-Modoc populations, the combined group is not significantly different from the Washington population, which is added at node B (Fig. 2). That is, six populations that supposedly belong to five different subspecies have minute differences in allele frequencies. Corbin (1983) found that the slope of the line generated from a plot of Nei's distance against F_{ST} in birds was steeper in intrageneric comparisons than in intraspecific comparisons. The slope of our line (0.109, Fig. 4) was lower than slopes for the intraspecific comparisons he surveyed, suggesting that we had sampled, on average, less differentiated populations than those in his sample.

The relative grouping of populations in Figure 2 highlights three of the California populations as being somewhat more distant genetically. Nei's distances generally exceeded 0.005 when CA-SF, CA-SAC, or CA-SALT were involved (Table 3), a value of D found to correspond to the distance between avian subspecies (Barrowclough 1980). It is interesting that California, with its topographical and ecological diversity, includes all or part of the ranges of 7 of 14 putative subspecies of Red-winged Blackbirds described for North America. Although our results do not support generally the number and location of Red-winged Blackbird subspecies currently accepted in the continental U.S. (AOU 1957), allele frequencies

among the California populations we sampled suggest significant subdivision in that geographical region.

It should be emphasized that the differences between these three California populations and populations from other locations were primarily due to differentiation at the Gr locus. Locus by locus F_{ST} values were significant ($P < 0.01$) at five loci (Table 2), but the G value was the highest for the Gr locus. However, gel resolution for the products of that locus were excellent, which supports the genetic validity of that locus. (Photographs of the Gr gels are available from the authors upon request.)

In spite of generally small genetic distances among populations in our sample, the F_{ST} value across all populations was high for avian conspecific populations surveyed thus far. Barrowclough and Johnson (1988) recently reviewed studies of 17 bird species for which F_{ST} values had been calculated. In their review, only the *Pipilo erythrophthalmus* complex ($F_{ST} = 0.229$) had a higher F_{ST} than our estimate for Red-winged Blackbirds ($F_{ST} = 0.165$), although the towhee complex may contain more than one biological species, resulting in an increased F_{ST} relative to conspecific comparisons. As stated earlier, our estimate of F_{ST} (0.165) was nearly four times larger when California populations were included in the analysis than when they were omitted.

Although Red-winged Blackbirds may move only a short distance during a given reproductive season (e.g. <25 km according to Dolbeer (1978)), it seems reasonable that individuals from a migratory population we sampled outside of California could mix with birds from other breeding areas during winter and return to breed in a different region the following year (AOU 1957, Burt and Giltz 1977, Dolbeer 1978, Knittle et al. 1987). Similarly, Red-winged Blackbird females from northern states (e.g. New York) may winter in Florida (James et al. 1984) where they could remain to breed with birds from the population we sampled there. That is, it is possible that gene flow is common enough among the non-California populations to prevent significant differences in allele frequencies from developing. Unfortunately, this suggestion is necessarily speculative because the quantitative details that would relate dispersal distances with genetic data do not exist.

The CA-SF and CA-SAC populations appar-

ently are resident year-round where we sampled them (Van Rossem 1926, AOU 1957), although "local" movements are common (Grinnell and Miller 1944). CA-SALT, which occurs well south of CA-SF and CA-SAC, is either resident (Grinnell and Miller 1944) or migrates farther south in winter (AOU 1957). The remaining California population we sampled (CA-MOD) migrates from its breeding area in winter, and could overlap with Red-winged Blackbirds from other breeding sites in California (AOU 1957). The genetic differences between Red-winged Blackbirds at CA-SAC and CA-SF is even more puzzling because there is no significant physical barrier to movement between these locations.

CA-SF and CA-SALT both breed in areas of brackish water, which could result in physiological adaptations that prevent or reduce interchange with populations living in upland or freshwater environments, such as CA-SAC. Basham and Mewaldt (1987) demonstrated a significant difference in salt tolerance between two subspecies of Song Sparrows (*Melospiza melodia*) on San Francisco Bay. The individuals that live on the bay were more tolerant than individuals captured several kilometers away from the bay, and Basham and Mewaldt (1987) hypothesized that this difference would prevent gene flow between the two populations. This hypothesis could be tested by examining physiological tolerance for salt water and genetic evidence for gene flow among populations of several species of birds found in salt- and freshwater environments in California. If selection is not responsible for differences between CA-SF/CA-SALT and CA-SAC, we can only attribute the results to genetic drift permitted by strong breeding-site fidelity.

Our results based on allozyme analysis are quite similar to the results of a study of Red-winged Blackbird mtDNA by Ball et al. (1988). In general, they found limited phylogenetic population structure and small genetic distances among populations. Ball et al. (1988) obtained samples in California only from *caurinus*, so we do not have a direct comparison between mtDNA and allozymes with respect to those birds in our study (CA-SF and CA-SALT) that were most different from other demes sampled. Sample sizes for the mtDNA ranged from 1 to 19 individuals ($\bar{x} = 6.7$) per breeding locality, which precludes any quantitative analysis based

on haplotype frequencies. The greatest differentiation in mtDNA among populations, however, involved the nonmigratory Red-winged Blackbirds sampled in Mexico (Ball et al. 1988).

The greater differences among populations in California relative to among-population differences in non-California populations suggest that no single genetic model of population structure or gene flow may apply to the entire species in North America. Isolation-by-distance, island, and stepping-stone models are the classic genetic models used to describe both the mechanisms of gene flow and the resulting structure among conspecific populations (Rockwell and Barrowclough 1987). Our data for the region outside California would seem to support an island model as defined by Rockwell and Barrowclough (1987). Mixing of individuals from different breeding areas on the wintering grounds, and the subsequent return of individuals (e.g. first-time breeders) to different breeding areas the following spring could produce gene flow via quantum geographic movement of genes from one generation to the next, unlike the slow diffusion implied by the isolation-by-distance model. This quantum movement of genes occurs in waterfowl (see Rockwell and Barrowclough 1987), because males pair on the wintering grounds and tend to follow the female to her previous breeding location.

Red-winged Blackbirds from our California sample sites are apparently less migratory than birds from our other sites. This behavior, coupled with possible selection for saline habitats, could reduce gene flow substantially. In California, an island or stepping-stone model would seem to apply. It is important to decide which genetic model—if any of the three—applies. Derivation of a rigorous model of gene flow (population structure) for this species depends on continued development of models based on empirical observation, which will require greater knowledge of adult movements and dispersal of young.

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