VOCAL DIALECTS AND GENE FREQUENCIES IN THE CHINGOLO SPARROW (*ZONOTRICHIA CAPENSIS*)

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Zonotrichia capensis, known in the literature as the Chingolo, Andean Sparrow, and Rufous-collared Sparrow, is one of four species of the genus *Zonotrichia*, all confined to the New World. The Chingolo is a small songbird that ranges from Tierra del Fuego to the Mexican state of Chiapas, and has also colonized the Caribbean islands of Santo Domingo, Aruba, and Curaçao. This species is distributed over 6400 km of latitude, and 4800 km of longitude, from sea level to 5000 m of altitude. Within this extraordinary range, Chapman (1940) recognized 22 races. The Argentine provinces of Buenos Aires and Tucumán, where the present study was conducted, fall within the range assigned by Chapman to *Z. capensis hypoleuca*.

Neighboring populations of *Z. capensis* frequently differ in the characteristics of their song. When these differences are marked and systematic they give rise to dialects (Nottebohm 1969). Over vast areas of pampas country, the dialect of the Chingolo remains unchanged. But where life-zones change over short distances, as in altitudinal transects, dialects also change over short distances. This observation suggests that song dialects in this bird may have evolved to reduce gene flow between neighboring populations experiencing different selective pressures. Females born in a given dialect area would breed in that dialect area and choose as mates males with a song that matched that dialect; males, in turn, would learn to sing the dialect of their birth area (Nottebohm 1969, 1970). To test this hypothesis, it is necessary to evaluate the genetic differences that may exist between neighboring populations with different dialects, and to compare them with differences between samples of neighboring populations having the same dialect. Measures of gene frequencies have been used in the past to evaluate the degree of gene flow between segments of rodent populations (Selander 1970; Selander et al. 1971), but the present report is a first attempt to correlate vocal and genetic differences among populations of a songbird species. Though the results presented here do not permit a final assessment of the influence of song dialects on gene flow, they constitute the first report of simultaneous measurements of allelic and dialect characteristics of avian populations.

MATERIALS AND METHODS

One of us (F. N.) visited Argentina from September to December 1969. Extensive recordings of the song of *Z. capensis* were made in the provinces of Buenos Aires and Tucumán. A detailed analysis of these sound recordings is in preparation and will appear as a complementary publication (Nottebohm and Nottebohm, in prep.). A smaller fraction of birds was recorded singing and then collected, and these individuals provide the material for the present report.

Z. capensis hypoleuca is a bird of open spaces and parkland vegetation, where it feeds on the ground. It is common in areas devoted to agriculture and grazing. Samples 1, 2, and 3 of this study were collected in such habitats in Buenos Aires Province, and sample 4 was collected some 1000 km to the northwest, in Tucumán Province (fig. 1).

Sample 1: 17 birds were collected on 26 and 27 October in the coastal lowlands near the town of Pinamar over an area some 20 km in diameter, at an altitude of 4 to 10 m above sea level.

Sample 2: 18 birds were collected at Estancia La Brava and environs, 130 km southwest of Pinamar on 2 and 3 November. These specimens were collected at the eastern tip of the highlands of Buenos Aires Province, at an altitude of 70 m above sea level, over an area some 8 km in diameter.

Sample 3: 22 birds were collected on 8 November at Estancia La Azucena, a locality 125 km northwest of La Brava, 220 km to the west of Pinamar, and 225 m above sea level. La Azucena is in the midst of the Sierras de Tandil, surrounded in all directions by highlands. This sample was collected over a radius of 5 km.

Sample 4: 20 birds were collected on the road ascending the Sierras de Aconquija, and linking Acheral, Tafí del Valle, and Infiernillo in the northwest part of Tucumán Province. Collections were made between 18 and 28 November, at the following altitudes: one bird at 610 m, one at 976 m, one at 1396 m, one at 1769 m, three at 1866 m, two at 1900 m, two at 2592

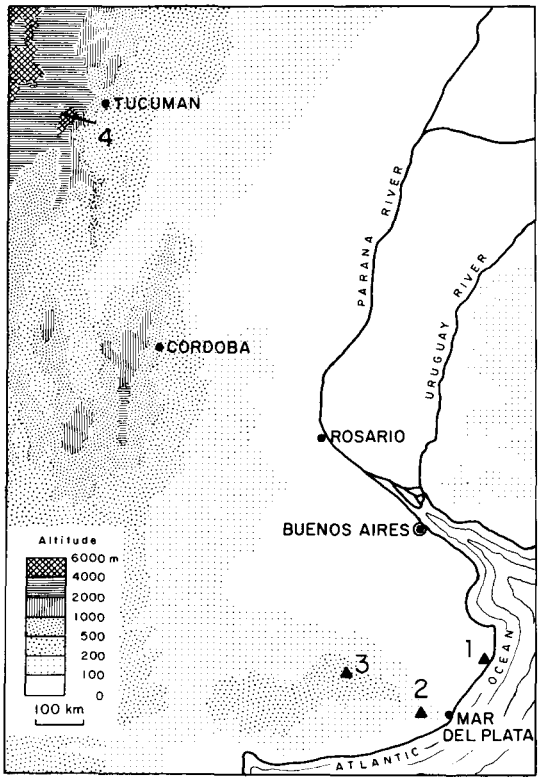

FIGURE 1. Partial map of Argentina and neighboring countries showing the location of the four areas sampled. Numbered black triangles correspond to the following localities: 1, Pinamar; 2, La Brava; 3, La Azucena; number 4 indicates the position of the Tucumán transect.

m, one at 2623 m, one at 2672 m, one at 2720 m, one at 2757 m, three at 2775 m, and one at 2873 m. This altitudinal transect covers a distance of 40 km, as the crow flies. By road this distance increases to 66 km, and one traverses *five successive life-zones*, as follows: from 550 m to 900 m, foothills subtropical forest; from 900 m to 1300 m, forest with predominance of trees belonging to the Myrtaceae family. Beyond 1300 m, the composition of the forest changes gradually, until between 1750 and 1850 m it is dominated by alders (*Alnus jorullensis*). Above 1850 m the terrain is open, with semi-desert vegetation represented by a sparse cover of grasses and shrubs. From 2200 m on and all the way up to Infiernillo, at 3042 m, the dominant grass is *Festuca hieronymi*, and shrubs disappear (Meyer and Weyrauch 1966).

Z. capensis is distributed continuously between sample localities 1, 2, and 3, and no physical barrier separates birds breeding in these three areas. The distribution of *Z. capensis* in sample area 4 is a bit more difficult to interpret. The open terrain between 1850 m and 3042 m is undoubtedly good Chingolo habitat, and the alder woodland between 1750 and 1850 m is probably also part of this species' native habitat. But it is doubtful whether the dense forest below 1750 m has ever been primary Chingolo habitat. In this life-zone, Chingolos are found along the grassy banks of the road and on man-made clearings. Below 550 m, the native woods have been turned into farmland. Chingolos in this latter area were studied by King (in press).

All birds in samples 1, 2, and 3 were males. Three birds in sample 4 were females, but only two of these females were recorded singing before they were collected.

In the areas of samples 1, 2, and 3, *Z. capensis* starts laying in late October and continues to do so into November (Hudson 1920; Wetmore 1926; Nottebohm and Nottebohm, in prep.). In area 4, onset of breeding varies with altitude. King (in press), working in the Tucumán area, found that maximal testicular development was achieved at 550 m by late October, and maximal ovarian and testicular development at 2000 m by December. Hence, some birds in sample 4 may not have reached their final breeding destination at the time they were collected.

Sound recordings were made with a Nagra III tape recorder and a Sennheiser MKH 804 directional microphone. Recordings were analyzed with a Kay Electric Co. 6061 B Sona-graph, with the "high shape" circuit and "wide band" filter setting.

Altitudes at which birds were collected were obtained from topographic maps from the Argentine "Instituto Geográfico Militar," supplemented by readings from a Kollsman C-12 aircraft altimeter.

The 77 birds collected were placed immediately in a container with dry ice, then stored at –20° C. These specimens were transported on dry ice to Austin, Texas. There R. K. S. prepared extracts of kidney, liver, and heart for use in starch-gel electrophoresis. Variation in some 23 proteins was assayed, using electrophoretic and staining techniques similar to those described in an earlier paper (Selander et al. 1971).

SONG DIALECTS

Song variation in *Z. capensis hypoleuca* has been described in an earlier publication (Nottebohm 1969). Over the areas surveyed, the song of this species lasts 1.0 to 2.0 sec. and falls between 3 and 7 kHz (fig. 2, 3). Chingolo songs are composed of a series of whistled notes varying in frequency modulation and length, followed by repetitions of a note, forming a trill. At any one locality the introductory part of the song varies among individuals, giving rise to different *themes*; the same individual may sing one to three different themes. By contrast, the trilled or second half of the song varies little among individuals from the same locality, regardless of the theme sung. This more stable part of the song indicates its *dialect* affiliation. Such a distinction between theme and dialect has biological significance. Dialects remain stable over hundreds of miles of homogeneous habitat, yet change from one life-zone to another. By contrast, some themes recur in neighboring life-zones. Thus, what reliably distinguishes neighboring populations breeding in different life-zones is their dialect, less so their themes. This notwithstanding, under some circumstances all or most birds in an area may sing the same dialect and theme. Such a predominance of a particular theme has been labeled a subdialect (Nottebohm 1969).

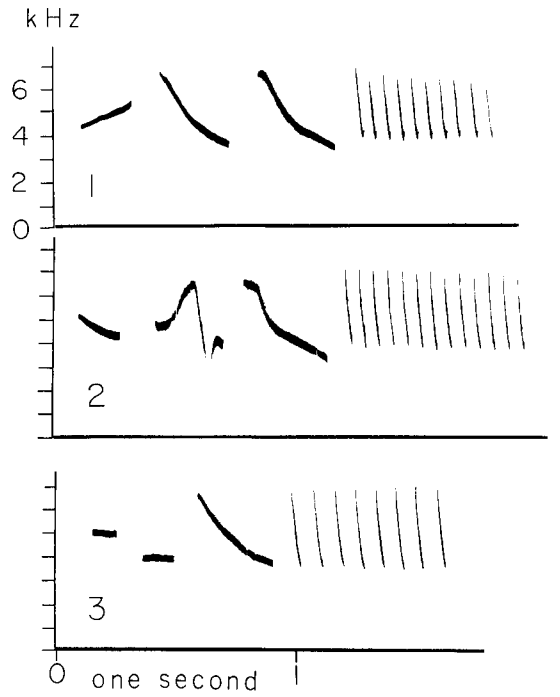

FIGURE 2. Song of three different Chingolo sparrows recorded, respectively, at Pinamar (1), La Brava (2), and La Azucena (3), on 26 October, 2 November, and 8 November 1969.

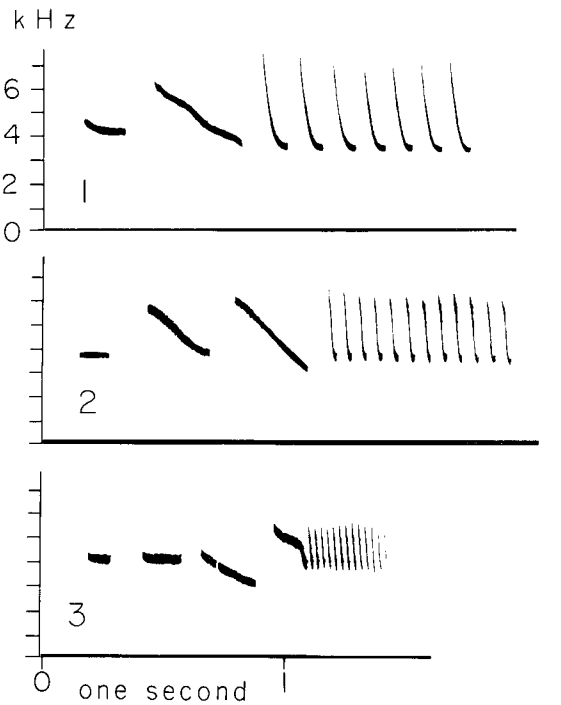

FIGURE 3. Song of three different Chingolo sparrows recorded in Tucumán on the Acheral-Tafí del Valle-Infiernillo transect (sample 4 in fig. 1). Recordings were made at 940 m (1), 2623 m (2), and 2873 m (3). Song (1) is representative of the *Alnus jorullensis* woodland dialect, which also extends to the lower altitude myrteceae woodland; (2) of the grassy slopes above Tafí del Valle; (3) of the high altitude Infiernillo population. Song 1 was recorded on 20 November 1969; 2 and 3 on 26 November 1969.

Samples 1 and 2 share the same song dialect characterized by a faster trill than that of sample 3 (fig. 2). Sample 4 includes birds with three different song dialects (fig. 3), and these were altitudinally arranged. Both in Tucumán and Buenos Aires provinces, song dialects were observed to grade into each other.

GENETIC VARIATION AS REVEALED BY PROTEIN POLYMORPHISM

A total of 23 enzymes and nonenzymatic proteins (encoded by 24 structural gene loci) was examined for allozymic variation, but only 15 proteins appeared with sufficient constancy and clarity to permit us confidently to score the phenotypes of all individuals in our samples. Excluded from further consideration in our analysis were the following eight proteins.

Esterase-1, Esterase-2, and Esterase-3. The most intensely staining esterase system (esterase-1) in liver and kidney extracts electrophoresed on lithium hydroxide gels (Buffer System 2; Selander et al. 1971) may be polymorphic, with at least four alleles, but consistent scoring was impossible, due in part to the fact that bands of another esterase system have mobilities similar to those of the esterase-1 system. The most anodal esterase (esterase-2) apparently is monomorphic, but unexplained irregularities in mobility of bands prevent our including this protein among the monomorphic group. Esterase-3, a slowly migrating anodal protein appearing weakly in kidney extracts electrophoresed on Buffer System 3, may also be monomorphic.

Peptidase-1 and *Peptidase-2.* Two peptidase systems appear on gels in which liver extracts were electrophoresed on a tris-citrate buffer (Buffer System 4) and stained with a solution containing the dipeptide leucine-alanine as substrate. The more anodal of these (peptidase-1) appears to be weakly polymorphic, with a total of three alleles, but the resolution of bands is rather poor; over 90% of the individuals are homozygous for a common allele. Peptidase-2, a weakly staining system migrating slower than peptidase-1, probably is monomorphic.

Phosphoglucomutase-3. Bands of the most anodal PGM system are irregular in mobility and staining intensity.

TABLE 1. Allele frequencies at two polymorphic loci in *Zonotrichia capensis*.

Sample locality	No. specimens	Phosphoglucomutase-1					Phosphoglucose isomerase-1		
		Pgm-1^a	*Pgm-1*^{b}	*Pgm-1*^{c}	*Pgm-1*^{d}	*Pgm-1*^{e}	*Pgi-1*^{a}	*Pgi-1*^{b}	*Pgi-1*^{c}
Pinamar	17		0.03	0.06	0.06	0.85	0.03	0.97	
La Brava	18			0.03	0.06	0.92	0.03	0.94	0.03
Tandil	22				0.02	0.98		1.00	
Tucumán	20	0.03		0.03		0.95	0.03	0.95	0.03

Isocitrate dehydrogenase-2. This weakly staining IDH, which presumably is the mitochondrial form, fails to migrate far from the origin (point of insertion of the extract in the gel). It is perhaps polymorphic.

Alcohol dehydrogenase-1. ADH activity is weakly represented in liver extracts by a cathodally migrating band (Buffer System 7). Apparently the protein is monomorphic, but in many individuals the band is faint or absent altogether.

Of the 15 proteins scored in all individuals, the following 10 (encoded by 11 loci) were monomorphic, no variants being detected. At each locus, a single allele appears to be fixed in all populations sampled.

Phosphoglucomutase-2 (liver; Buffer System 7). This system migrates a short distance anodally and stains very intensely.

Glutamate oxalate transaminases (liver; Buffer System 5). Two GOT systems, presumably representing the supernatant (GOT-1) and mitochondrial (GOT-2) forms, are represented. On Buffer System 6, GOT-1 moves a short distance anodal to the origin and GOT-2 migrates far cathodally and shows several poorly resolved conformational bands.

Malate dehydrogenases (kidney or heart; Buffer System 4). Two intensely staining NAD-dependent MDH systems appear, an anodal form (MDH-1) and a cathodal form (MDH-2), presumably representing, respectively, supernatant and mitochondrial forms.

Lactate dehydrogenase (kidney and heart; Buffer System 3). This enzyme, the active unit of which is a tetramer, is encoded by two loci, *Ldh-1* and *Ldh-2*. Both loci are monomorphic in the samples examined, all individuals having a five-banded phenotype.

Albumin and three other nonenzymatic proteins (liver; Buffer System 2). Four prominent anodally migrating bands appear in liver extracts stained with the "general" protein stain amido black. All are unusually well resolved and all are monomorphic. The most anodal of the four is albumin. (Additionally, we can distinguish two weakly staining proteins, one anodal to the albumin and one just anodal to the origin; and both appear to be similarly invariant.) We have considered the four prominent proteins to be encoded by separate loci, designated, in order of decreasing mobility, *Alb-1*, *Pt-A*, *Pt-B*, and *Pt-C*.

Five of the proteins examined are polymorphic in one or more of the four samples studied, as follows.

Phosphoglucomutase-1 (liver; Buffer System 7). Migration is cathodal, and five alleles are represented, as shown in table 1. (At this and other polymorphic loci, alleles are designated alphabetically in order of increasing mobility of their respective allozymes.) Heterozygotes are double-banded.

Phosphoglucose isomerase-1 (liver; Buffer System 7). On the phosphate buffer employed in this study, three alleles are represented (table 1); bands representing the *Pgi-1*^{a} and *Pgi-1*^{b} alleles migrate cathodally, while that corresponding to the *Pgi-1*^{c} allele moves anodally. Heterozygotes have a third band of intermediate mobility.

6-Phosphogluconate dehydrogenase-1 (liver; Buffer System 9, with NADP added to gel). Three alleles are represented (table 2). Homozygotes are single-banded and heterozygotes

TABLE 2. Allele frequencies at two polymorphic loci in *Zonotrichia capensis*.

Sample locality	No. specimens	6-Phosphogluconate dehydrogenase-1			α-Glycerophosphate dehydrogenase-1	
		6-Pgd-1^a	*6-Pgd-1*^{b}	*6-Pgd-1*^{c}	*α-Gpd-1*^{a}	*α-Gpd-1*^{b}
Pinamar	17	0.09	0.91		0.03	0.97
La Brava	18	0.17	0.83		0.06	0.94
Tandil	22	0.25	0.73	0.02	0.07	0.93
Tucumán	20	0.08	0.93		0.05	0.95

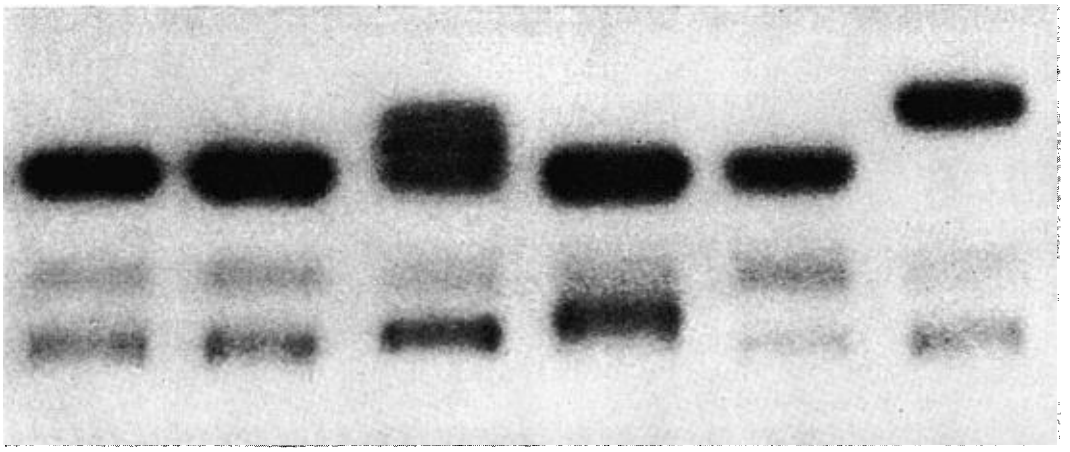

FIGURE 4. Electrophoretic pattern of 6-phosphogluconate dehydrogenase in *Zonotrichia capensis* (composite extracts of kidney and heart on tris-maleic-EDTA gel, pH 7.4). Darker upper system is 6-PGD-1, double-banded lighter lower system represents malic enzyme activity. Presumed genotypes at the *6-Pgd-1* locus (reading from left to right) are as follows: *b/b, b/b, a/b, b/b, b/b,* and *a/a.*

triple-banded, as shown in figure 4. Allele frequencies are shown in table 2, and observed and expected proportions of the various genotypes are presented in table 3.

α-Glycerophosphate dehydrogenase-1 (liver; Buffer System 9). This enzyme is polymorphic for two alleles in each of the samples examined (table 2). Heterozygotes have three bands.

Isocitrate dehydrogenase-1 (kidney; Buffer System 7). This IDH, which presumably is the supernatant form, migrates cathodally. Only a single variant individual was detected; this bird, from sample 2, is heterozygous for the common allele (*Idh-1*^{b}) and an allele (*Idh-1*^{a}) producing a faster migrating band. The frequency of the *Idh-1*^{a} allele in the La Brava sample is 0.03.

GEOGRAPHIC VARIATION IN ALLELE FREQUENCIES

At each of the five polymorphic loci, one allele is predominant in all parts of the range sampled. Excluding from consideration for the moment the Tucumán sample, we may say the following about intersample variation in allele frequencies. At three loci (*6-Pgd-1, Pgm-1* and *α-Gpd-1*), there is clinal variation from Pinamar to La Brava to Tandil. Thus, for example, at the *6-Pgd-1* locus, the frequency of the *6-Pgd-1*^{a} allele increases clinally from 0.09 at Pinamar to 0.25 at Tandil (table 2). Although for none of the loci can we demonstrate heterogeneity in allele frequencies at conventionally accepted levels of probability (e.g., for *6-Pgd-1*, $\chi^2_{(2)} = 3.69$, $P > 0.10$ for the three localities, omitting the *6-Pgd-1*^{c} allele), the fact that the observed changes in allele frequencies at three of the five loci are geographically oriented suggests that real interpopulation variation is reflected in the frequencies represented in our small samples. The observed variation at the *Pgi-1* and *Idh-1* loci is too restricted and does not lend itself to clinal interpretation.

For the three loci (*6-Pgd-1, α-Gpd-1,* and *Pgm-1*) showing clinal variation, the allele frequencies in the La Brava sample fall close to the midpoint between extremes represented by the material from Pinamar and Tandil. Since La Brava is roughly equidistant between Pinamar and La Azucena (fig. 1), variation in the degree of genetic similarity among these

TABLE 3. Genotypic proportions at the *6-Pgd-1* locus in *Zonotrichia capensis.*

Sample locality	No. individuals	Genotype: observed (expected)[a]					
		a/a	*b/b*	*c/c*	*a/b*	*a/c*	*b/c*
Pinamar	17	0(0.1)	14(14.1)		3(2.8)		
La Brava	18	1(0.4)	13(12.4)		4(5.1)		
Tandil	22	2(1.3)	12(11.5)	0(0.0)	7(8.2)	0(0.3)	1(0.7)
Tucumán	20	1(0.1)	18(17.1)		1(2.8)		

[a] Expected numbers calculated by the unbiased method of Levene (1949) for small samples, assuming Hardy-Weinberg equilibrium.

three samples might be attributed to the distances separating them. Yet this in itself is not an explanation. A cline as observed could reflect restrictions in gene flow between potentially panmictic populations, could result from an altitudinal gradient in selective factors, or could be the outcome of an interaction between these two factors.

The allele frequencies at each of the five polymorphic loci in sample 4 are remarkably similar to those of samples 1, 2, and 3 (tables 1, 2). Sample 4 was collected over an altitudinal span of almost 2250 m, and over a distance of 40 km. On the assumption that fitness of different alleles varies according to life-zone, we might have expected this sample to be more heterogeneous and to differ significantly from the Buenos Aires samples. However, the number of individuals collected per life-zone in sample 4 is too small to permit a confident interpretation of the homogeneity revealed by this sample. Also, the difference in environments of the Tucumán and Buenos Aires populations may be less dramatic if we consider that the higher altitude populations in Tucumán are probably migratory.

GENIC HETEROZYGOSITY

The level of genetic variability in a population is conveniently expressed in terms of the average proportion of loci in heterozygous state (H) in individuals. For the four samples of *Z. capensis*, estimates of H, derived from allele frequencies, are as follows: Pinamar, 3.4%; La Brava, 4.6%; Tandil, 3.1%; and Tucumán, 3.0%. The mean heterozygosity is 3.53%. This value is lower than that estimated for the house mouse (*Mus musculus*) ($H = 6.27\%$ for 32 nonesterase loci analyzed in a population at Hallowell Farm, California; Selander and Yang 1969) but within the range of values recorded for populations of the old-field mouse (*Peromyscus polionotus*) (H varies from 3.40% in South Carolina and Georgia to 5.93% in peninsular Florida; Selander et al. 1971). Comparable estimates are not presently available for other species of birds.

VOCAL DIALECTS RE-EVALUATED

The genetic cline observed between the lowlands and highlands of Buenos Aires Province might have been predicted from the observation that the dialects from these two life-zones grade into each other. Female choice of mate probably includes birds deviating a little toward either end of the dialect gradient. Thus, gene flow in *Z. capensis* probably does not stop at any one point between contiguous segments of neighboring populations. This is not to say that dialects play no role in the emergence of local adaptations. They may well discourage the saltatory dispersal of genes as would occur if a male from one dialect population were to breed with a female born in the center of a neighboring or further removed dialect population. Avian dialects may reduce gene flow between adjacent populations to rates such as we might find in many land mammals. A rigorous test of this interpretation will require not only more sensitive sampling techniques but also a measure of the survival value of allelic differences between wild populations of the same species.

CONCLUSIONS

In the present study, we have explored the manner in which electrophoretically demonstrable allelic variation can be used to investigate the role of behavior, and in particular vocal dialects, in restricting gene flow among populations and, hence, producing genetic subdivision or deviation from panmixia (King 1967; Selander 1970). However, it is obvious that additional polymorphic loci and larger samples of individuals will be required for a definitive analysis of the major problem at hand. Samples of 30 to 50 individuals from a given locality or life-zone are needed in order to obtain reliable estimates of frequencies of the less common alleles.

Within local collecting areas, our evidence suggests that the population is essentially panmictic, but additional work is required to determine the extent of heterogeneity in allele frequencies among local populations of a single dialect. Unless we have good estimates of *local* heterogeneity, we are apt to confound intradialect and interdialect variation.

Despite their incomplete nature, the observations reported here throw new light on what exactly song dialects might accomplish. If dialects can be used to discriminate between contiguous and saltatory gene flow, their biological significance would seem well justified.

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