

GENETIC VARIABILITY IN CORY'S SHEARWATER (*Calonectris diomedea*)

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ABSTRACT.—Three different subspecies are recognized for Cory's Shearwater (*Calonectris diomedea diomedea*, *C. d. borealis*, and *C. d. edwardsii*). This long-lived colonial procellariiform shows an east-west cline in several morphometric characters. A strong colony and site fidelity has been reported, which suggests genetic isolation among colonies and low levels of gene flow. Three different colonies, including two subspecies (*C. d. borealis*, from the Azores, and *C. d. diomedea*, from Sardinia and Sicily), were sampled to investigate the genetic structure of the population. Six polymorphic loci were found in 36 tested. The percentage of polymorphism (P) and average heterozygosity (H) were $\bar{P} = 0.139$ and $\bar{H} = 0.027$, respectively. There were differences among colonies. Three of the polymorphic loci (PEP-A, PGM-1, and Pt5) showed significant values for F_{ST} (mean "unbiased" $F_{ST} = 0.083$). We found low values of heterozygosity and rare alleles for the other three (EST-2, G6PD, and GSR). Gene flow, estimated with Wright's and Slatkin's approaches, yielded values of Nm that ranged from 4.33 to 19.45 individuals exchanged among colonies per generation. Nonetheless, the subspecies are separate genetically, as shown by UPGMA dendrograms of Nei's and Rogers' genetic distances. The average genetic distance between the two Mediterranean and the Atlantic colonies is $\bar{D} = 0.006$, the threshold value reported between subspecies in birds. Accepting the existence of gene flow among colonies, the morphological cline may be related to environmental variables, but an interaction of selection and gene flow might also account for the observed genetic differences. Received 12 April 1988, accepted 8 February 1989.

THREE different subspecies of Cory's Shearwater are recognized: *Calonectris diomedea diomedea*, restricted to the Mediterranean; *C. d. borealis*, breeding in the subtropical islands of the East Atlantic (Azores, Desertas, Selvage, and Canary islands); and *C. d. edwardsii*, endemic to the Cape Verde Islands (Cramp and Simmons 1977). Body size is the main difference among the subspecies. *C. d. borealis* is larger than *C. d. diomedea*. *C. d. edwardsii* is the smallest with a thinner, blackish bill, less yellow than the other subspecies (Cramp and Simmons 1977). Different vocalizations have been recorded between *C. d. edwardsii* and *C. d. borealis* (Bannerman and Bannerman 1968).

The Atlantic and Mediterranean populations differ morphologically (Ristow and Wink 1980, Borg and Cachia Zammit pers. comm., Iapichino et al. 1983, Araujo et al. 1976, Witt et al. 1984). A dimensional trend occurs among the different Mediterranean colonies (Fig. 1, Table 1). The east-west cline might be explained partly by environmental variables such as differences in food availability linked to variations in productivity of different areas within the basin (Massa and Lo Valvo 1986). The cline may have

a genetic origin. The breeding of monogamous and philopatric pairs with high colony fidelity may lead to greatly reduced gene flow among colonies (Cachia Zammit and Borg 1987, Wink et al. 1987).

We used an electrophoretic analysis of blood proteins to test whether the subspecific taxonomic subdivision between *borealis* and *diomedea* can be supported on a genetic basis, and to analyze the genetic structure of the colonies in relation to the presumed absence of gene flow.

MATERIALS AND METHODS

Blood samples ($n = 31$) of *C. d. borealis* from São Miguel and Vila Franca islands in the Azores were obtained in July 1986. One hundred samples of *C. d. diomedea* from the colony breeding on Linosa Island were collected during April 1986. Another small colony of *C. d. diomedea* off the southwestern coast of Sardinia yielded 14 samples (Fig. 1).

All the birds were caught near the nest, banded, measured, and weighed. Using syringes, we drew 1 ml of blood by venipuncture from the brachial vein on the ventral side of the humerus. About 0.1 ml of 2.7% Na₂ EDTA solution was added to each sample. Plasma and red blood cells were separated by cen-

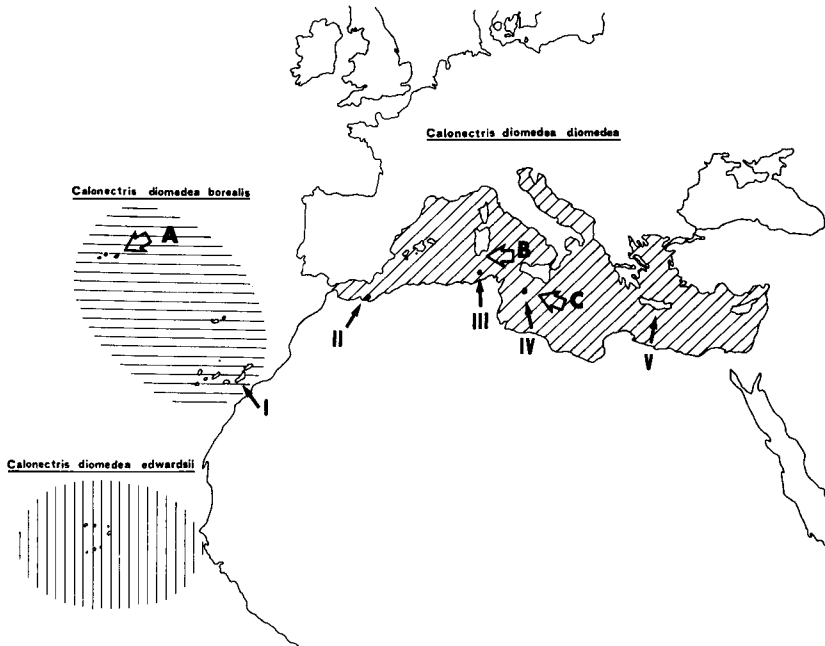


Fig. 1. Ranges of three subspecies of *Calonectris diomedea* with collecting sites (A = Azores, B = Sardinia, C = Sicily) and location of colonies in the Mediterranean and Atlantic (I = Great Selvage, II = Chaffarinas, III = Zembra, IV = Linosa, V = Aegean Sea).

trifugation and stored at -80°C until electrophoresis. Vertical polyacrylamide gel (VPAGE) and cellulose acetate membrane (CAM) electrophoresis resolved a total of 36 putative loci (Appendix).

The individual banding patterns were used to calculate the allele frequencies, the percentage of polymorphic loci (P), the observed and expected (H_{exp}) single locus heterozygosity (Ferguson 1980). We tested agreement of observed and expected heterozygote frequencies by the Hardy-Weinberg distribution by a Chi-square test (Li and Horvitz 1953). The genetic population structure was analyzed by the methods of Nei (1977) and Wright (1978), and with the F -statistic estimators of Weir and Cockerham (1984). We used Chi-square (Waples 1987) to test the significance of

the F_{IS} and F_{ST} estimates. Nei (1972) and Rogers (1972) genetic distances were used to make UPGMA dendrograms (Sneath and Sokal 1973) of relationships among colonies. Estimates of gene flow were made with the qualitative and quantitative approaches as proposed by Slatkin (1981; 1985a, b; 1986), as well as Wright's model (1978).

RESULTS

We found 6 polymorphic loci with 2 alleles each out of a total of 36 (Table 2). The average percentage polymorphism (at the 0.01 level) was $\bar{P} = 0.139$ (the GSR locus showed a rare allele

TABLE 1. Morphometric measurements (mean) in 6 colonies of Cory's Shearwater.^a (Locations in Fig. 1.)

	Mass (g)		Wing (mm)		Tarsus (mm)		Bill length (mm)		Bill depth ^b	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
I. Great Selvage	955	817	371	363	60.2	58.1	58.1	55.7	17.1	15.3
II. Chaffarinas	717	622	361	351	56.8	54.1	52.2	48.7	14.7	12.1
III. Zembra	703	575	355	343	—	—	50.7	46.9	13.8	12.1
IV. Linosa	675	577	356	345	53.6	52.8	53.6	50.6	13.7	12.0
V. Aegean Sea	587	514	342	333	53.4	52.0	49.5	46.2	12.9	11.6

^a Values for Chaffarinas, Zembra, Linosa, and Aegean Sea, and for the bill depth of the Great Selvage, are from Lo Valvo and Massa (1988); other values for the Great Selvage, from Robertson and James (1988).

^b Excludes nostrils.

TABLE 2. Allele frequencies and expected single locus heterozygosity (H_{exp}), in three colonies of Cory's Shearwater. Sample sizes were 100 (Sicily), 14 (Sardinia), and 31 (Azores). Values in parentheses are SE.

Locus ^a	Allele	Allele frequency			Expected heterozygosity ^b		
		Sicily	Sardinia	Azores	Sicily	Sardinia	Azores
PEP-A	a	0.480	0.464	0.833	0.50	0.50	0.28*
	b	0.520 (0.035)	0.536 (0.094)	0.167 (0.048)			
PGM-1	a	0.245	0.179	0.448	0.37	0.29	0.49
	b	0.755 (0.031)	0.821 (0.072)	0.552 (0.065)			
Pt5	a	1.000	0.964	0.968	0.00	0.07	0.06
	b	0.000 (0.000)	0.036 (0.035)	0.032 (0.022)			
EST-2	a	0.980	1.000	1.000	0.04	0.00	0.00
	b	0.020 (0.010)	0.000 (0.000)	0.000 (0.000)			
G6PD	a	0.010	0.000	0.017	0.02	0.00	0.03
	b	0.990 (0.007)	1.000 (0.000)	0.983 (0.016)			
GSR	a	0.005	0.000	0.000	0.01	0.00	0.00
	b	0.995 (0.005)	1.000 (0.000)	1.000 (0.000)			

^a See Appendix.

^b The agreement with the expected Hardy-Weinberg equilibrium was tested using the Li and Horvitz's (1953) formula. * = $P < 0.01$.

in the Sicily sample with a frequency < 0.01). The average expected heterozygosity was $\bar{H}_{exp} = 0.027$. There were no differences (99% confidence interval of the values) among colonies for \bar{H}_{exp} (Azores = 0.024, Sardinia = 0.024, Sicily = 0.026) and \bar{P} (Azores = 0.111, Sardinia = 0.083, Sicily = 0.111). Only the PEP-A locus in the Azores sample showed a significant departure from the expected Hardy-Weinberg equilibrium (Table 2).

We divided the polymorphic loci into two sets. PEP-A and PGM-1 had high values of total genetic diversity (H_{tot}) and EST-2, G6PD, GSR and Pt5 had low H_{tot} values (Table 3). The 3 colonies have significantly different allele frequencies at the PEP-A, PGM-1, and Pt5 loci. Almost no difference among the colonies was contributed by EST-2, G6PD and GSR loci, though the allelic variants of these loci were distributed unevenly among the 3 samples (Table 3).

The proportion of total genetic diversity distributed among colonies (G_{ST}) shows single locus values very similar to the F_{ST} computed through Wright's (1978) formula (the "common explicit computational formula"; Weir and Cockerham 1984). To account for sampling biases, we used the estimators of Weir and Cock-

erham to obtain another set of F -statistics. Sampling biases (number of sampled colonies, mean sample size) were fairly large as F_{ST} "unbiased" values were about 40% larger than the "biased" ones (Table 3). However, G_{ST} , F_{ST} "common formula," and F_{ST} "unbiased" values were concordant and showed statistically significant heterogeneity among colonies at the PEP-A, PGM-1, and Pt5 loci (Chi-square test; Workman and Niswander 1970, Waples 1987).

F_{IS} estimates departure from random mating within colonies with significant negative values in case of heterozygous excess. No statistically significant F_{IS} value was observed (Table 3).

The average genetic distance (Nei 1972) between the Azores and the two Italian colonies was ca. 0.006 (Table 4), near the mean value of genetic distance among avian subspecies ($\bar{D} = 0.0066$; Corbin 1983). No significant genetic distance difference was observed between the Italian colonies. The UPGMA dendrogram computed from Nei's D did not discriminate the colonies. Roger's D indicated a slight difference (Fig. 2).

The qualitative estimate developed by Slatkin (1981) produced a pattern expected for species with high gene flow among local populations

TABLE 3. Analyses of the genetic population structure: total genetic diversity (H_{tot}), proportion of the total genetic diversity distributed among the colonies (G_{ST}), and F -statistic single locus values.^a

Locus	H_{tot}	G_{ST}	"Biased"		"Unbiased"	
			F_{ST}	F_{IS}	F_{ST}	F_{ST}
PEP-A	0.495	0.085	0.084**	0.136	0.151**	
PGM-1	0.404	0.040	0.039**	0.158	0.063**	
Pt5	0.028	0.021	0.023*	-0.024	0.035**	
EST-2	0.020	0.000	0.006	-0.010	0.009	
G6PD	0.020	0.000	0.002	-0.002	0.003	
GSR	0.006	0.000	0.002	0.005	0.002	
Mean	0.162	0.025	0.026	0.044	0.044	

^a Sources: H_{tot} and G_{ST} (Nei 1977); "biased" F_{ST} (Wright 1978); F_{IS} and "unbiased" F_{ST} (Weir and Cockerham 1984). The significance of F_{IS} and F_{ST} values were computed according to the Chi-square formulations given by Waples (1987); (* = $P < 0.05$, ** = $P < 0.01$).

(Fig. 3). The Slatkin algorithm (1985a) is as follows: $\ln \cdot p(1) = a \cdot \ln \cdot Nm + b$, where $a = -0.505$, $b = -2.44$, $p(1)$ is the mean frequency of "private alleles" in the sample ($p(1) = 0.125$ in our case). It yielded an estimated rate of gene flow $Nm = e - [(\ln \cdot p(1) - 6) / -a] = 46.78$. The mean sample size (48) requires a correction (Slatkin 1985a). We divided the estimate of Nm by the ratio of the actual sample size to 25. The corrected Nm mean value was 24.36, much higher than the limit $Nm = 10$, and we considered the population to be panmictic. Subsequently, Slatkin and Takahata (1985) noted that the proposed model may produce Nm values overestimated by roughly 20%. Including that correction factor in our calculations, we obtained $Nm = 19.45$, still a relatively high value.

Nm can also be estimated by Wright's (1978) formula: $F_{ST} = 1 / (4Nm + 1)$, based on the distribution of genetic heterogeneity among demes, mainly due to conspicuous polymorphisms. Wright's model is effective in the case of an infinite island two-dimensional population structure at equilibrium. When $Nm > 1$, it indicates panmixia. The proper estimation of F_{ST}

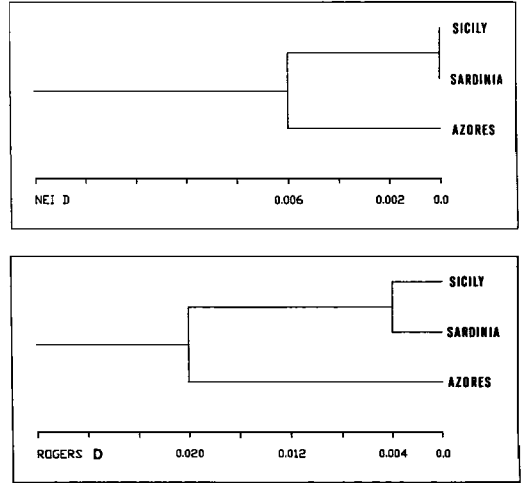


Fig. 2. UPGMA dendrograms computed from Nei's (1972) and Rogers' (1972) genetic distances.

is controversial and different computational methods have been suggested (Weir and Cockerham 1984). Consequently different values of Nm can be obtained (Table 5). Such values range from $Nm = 9.36$ to $Nm = 4.33$, well below $Nm = 19.45$ (Slatkin's model), but yet higher than 1, which indicates gene flow among Shearwater colonies.

DISCUSSION

The three Cory's Shearwater colonies did not have significantly different values of heterozygosity or percentage of polymorphism. A sample size effect presumably lowered the P value for Sardinia. Both \bar{P} and \bar{H}_{exp} in Cory's Shearwater were lower than mean values in other birds ($\bar{P} = 0.222$, $\bar{H}_{exp} = 0.053$; Corbin 1983). Genetic variability was distributed unevenly among colonies and mean F_{ST} value was above the mean values for geographic populations in birds ($\bar{F}_{ST} = 0.022$; Barrowclough 1983).

TABLE 4. Genetic distances (upper right) and identities (lower left) computed following Nei (1972) and Rogers (1972).

Population	Nei's			Rogers'		
	1	2	3	1	2	3
1. Sicily	—	0.000	0.005	—	0.004	0.017
2. Sardinia	1.000	—	0.006	0.996	—	0.018
3. Azores	0.995	0.994	—	0.938	0.982	—

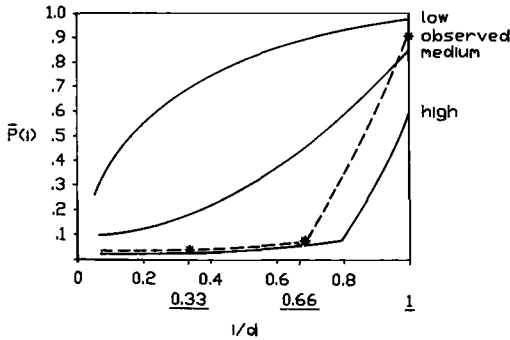


Fig. 3. Conditional average allele frequency $\bar{p}(i)$ plotted against incidence i/d for three colonies of *Calonectris diomedea*. $\bar{p}(i)$ is the average frequency of an allele in the populations in which it occurs; i/d is the ratio between the number of populations in which the allele i occurs and the total number (d) of sampled populations.

UPGMA dendrograms based on genetic distances separated the Mediterranean populations (Sicily and Sardinia) from the Atlantic colony (Azores). Nei's genetic distance among these populations was well within the mean values recorded among subspecies in birds. We assume that the taxonomic division between North Atlantic (*C. d. borealis*) and Mediterranean (*C. d. diomedea*) populations has a genetic basis.

Remarkable colony and site fidelity are reported in Shearwaters (Jouanin et al. 1977, Wink et al. 1987), though more data are needed for a proper demonstration of such behaviors (Cachia Zammit and Borg 1987). Notwithstanding, the colonies seem not to be isolated genetically. Our analysis of gene flow indicates panmixia. Perhaps 4–19 individuals could be exchanged among colonies per generation.

There are some potential difficulties in a proper interpretation of these data. The Nm estimate is dependent only on the migration rates among demes, and is relatively unaffected by the action of natural selection on single loci, geographical structure of demes, or sample size. The number of "private alleles" found within the sample is crucial. We found only 4 rare and 2 private alleles (EST-2, allele b; GSR, allele a). Furthermore above $Nm = 10$, the relationship between $\ln Nm$ and $\ln p(1)$ is no longer linear, and the Nm estimate is no longer quantitative. Private alleles were found only in the Sicily colony. We cannot exclude that a proportion-

TABLE 5. Values of F_{ST} and Nm obtained following different computational methods.^a

	"Biased" F_{ST}	"Unbiased" F_{ST}	G_{ST} ^c
F_{ST}	0.026	0.044	0.025
Nm	9.36	5.43	4.33

^a Sources: "Biased" F_{ST} (Wright 1978); "Unbiased" F_{ST} (Weir and Cockerham 1984).

^b G_{ST} is assumed to be equivalent to F_{ST} if the number of sampled populations is high, otherwise: $G_{ST} = 1/(4Nm \cdot a + 1)$, with $a = [n/(n-1)]^2$, and $n =$ number of sampled populations (in this case: $n = 3$, $a = 2.25$) (Slatkin 1985a).

ately larger sampling might allow us to find such alleles in the other two sites.

Wright's model requires an infinite island two-dimensional model, which might fit for the long-range dispersal during the spring movements of the wintering population towards the colonies. If mixed flocks build up in the Atlantic, it is likely that individuals (or pairs) of *C. d. borealis* may enter the Mediterranean, and *C. d. diomedea* individuals may stay and breed in the Atlantic (the probabilities for such "mistakes" being in principle the same). Recently, an individual banded as a nestling in an Atlantic colony (Great Selvage) 9 yr previously was found during the breeding season in Linosa (Mediterranean) (Lo Valvo and Massa 1988). Short-range linear dispersal movements might exist in young birds from a colony center, along the coasts of the islands, or towards different sites. On a microgeographic basis, this stepping-stone model might be more suitable (Slatkin 1985b).

It is unlikely that the Mediterranean population of Cory's Shearwater is at equilibrium, either demographically (e.g. from drastic and repeated crises due to human predation to the colonies; Jouanin et al. 1977, Mougouin et al. 1984) or genetically. Nei's (1975) model: $I = m/(m + v)$ estimates the maximum possible migration rate (m) among local populations, given average genetic identity among populations (I) and mutation rate (v). Because the actual mutation rate is not known, an average value of $v = 1.2 \times 10^{-6}$, can be used (Nei 1975). With $I = 0.995$ (Table 4), $m = 4 \times 10^{-4}$. This value allows evaluation of the local effective population size (N) starting from the estimated Nm ($N = Nm/m$) (Slatkin 1985a). If we take into account the values of Nm (Table 5), N values range from ca. 10,000–23,000 individuals. No direct estimate of

N exists for Cory's Shearwater, but the figures obtained compare favorably with colony-size data (Massa and Lo Valvo 1986). Nm may be considered at equilibrium after a number of generations (T) related to the migration rate (m). In our case: $T = 1/m = 2,500$ generations (Slatkin 1985a). If we assume an average of 6 yr as the age of first breeding in Cory's Shearwater (Zino 1971), 15,000 yr would be required to reach equilibrium. As colonization of Mediterranean islands is a consequence of the events that occurred during the Pleistocene (Voous 1974) ca. 10,000 yr BP (end of the last glacial period, Würm), it is possible Nm has not yet reached the equilibrium value.

We cannot distinguish between a situation of high gene flow or permanence of rare alleles within the populations as a consequence of dispersal from a common center of origin (radiation model; Slatkin 1985a). The probability of losing rare alleles because of random drift becomes high after $T = N$ yr, which corresponds to a number of generations ranging from $23,000/6 = 33,833$ to $10,000/6 = 1,667$. Because of Pleistocene colonization, a maximum of $10,000/6 = 1,667$ generations have been completed so far. Under these conditions the effects of gene flow become clearly detectable only if the average effective population size was lower than $N = 10,000$ since the Mediterranean colonization.

If gene flow exists among colonies, then environmental variables might play a role in maintaining the morphological cline. The observed genetic differences between the Atlantic and Mediterranean colonies could be explained by the interaction of gene flow and natural selection. If an allele is subjected to positive selection in a local population, then different alleles migrating at a rate m cannot prevent diversification when $m/s < 1$ (Nagylaki 1975). A coefficient of selection as low as $s = 4 \times 10^{-4}$ would be enough to effectively balance gene flow.

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APPENDIX. Electrophoretic conditions and loci (abbreviation, EC number, number of putative loci and electrophoretic method: VPAGE = vertical polyacrylamide gel; CAM = cellulose acetate membrane).

Monomorphic loci: adenylate kinase (AK, EC 2.7.4.3, 2 loci, CAM); α -naphthyl-acetate esterase-1 (ES, EC 3.1.1.1, 1 locus, VPAGE); hemoglobin (Hb, 3 loci, VAPGE); isocitrate dehydrogenase (IDH, EC 1.1.1.42, 2 loci, CAM); lactate dehydrogenase (LDH, EC 1.1.1.27, 2 loci, VPAGE); malate dehydrogenase (MDH, EC 1.1.1.37, 2 loci, VPAGE); leucyl-glycyl-glycine peptidase, leucyl-proline peptidase (PEP, EC 3.4.11, 2 loci, VPAGE); phosphoglucose isomerase (PGI, EC 5.3.1.9, 1 locus, CAM); 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44, 1 locus, CAM); peroxidase (POX, EC 1.11.1.7, 2 loci, VPAGE); superoxidodismutase (SOD, EC 1.15.1.1, 2 loci, VPAGE); serum proteins (Pt, 9 loci, VPAGE).

Polymorphic loci: glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49, 1 locus, VPAGE); glutathione reductase (GSR, EC 1.6.4.2, 1 locus, CAM); α -naphthyl-acetate esterase-2 (ES, EC 3.1.1.1, 1 locus, VPAGE); leucyl-alanine peptidase (PEP-A, EC 3.4.11, 1 locus, VPAGE); phosphoglucomutase-1 (PGM-1, EC 5.4.2.2, 1 locus, VPAGE); serum protein-5 (P5, 1 locus, VPAGE).

The electrophoretic methods and the staining recipes were modified from Harris and Hopkinson (1976), Davis (1964), and Meera-Khan et al. (1982).