Virginia Journal of Science
Volume 45, Number 1
Spring 1994

Gene Flow Among Island Populations of Marsh Rice Rats (*Oryzomys palustris*)

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ABSTRACT

Inter-island movement data and allelic frequencies were compared to assess the differences between direct and indirect estimates of gene flow for marsh rice rats on the Virginia barrier islands. Inter-island movements among four adjacent islands and the mainland were observed during the summer of 1989. Ten movements between two islands separated by 50 m and one movement between two islands separated by 300 m were documented. The direct estimate of dispersal for the five sites was 0.75 migrants per generation. Electrophoretic variation in proteins encoded by 13 presumptive gene loci was analyzed using blood samples collected from rats trapped at the five sites. The indirect estimate of migration using Wright's F_{ST} method was 0.09 migrants per generation. The differences in the direct and indirect estimates may be due to sampling error, lack of successful reproduction by the immigrants, or to differences in the time scales that the direct and indirect methods represent.

Key words: gene flow, genetic differentiation, *Oryzomys palustris*

INTRODUCTION

Populations of small mammals on islands are well suited to study genetic differentiation (Calhoun and Greenbaum, 1991; Navarro and Britton-Davidian, 1989; Hanski and Kuitunen, 1986; Gill, 1980; Berry et al., 1978). Genetic differentiation is generally estimated by measuring morphologic and/or or biochemical variation and then population patterns are interpreted using theories on natural selection, mutation, genetic drift and gene flow. Although biogeographical information and data on the biology of the species aid in these interpretations, few studies (Ehrlich and Raven, 1969) have attempted to combine the genetic data with data on individual movements.

Gene flow within a species determines the extent to which genetic changes in local populations are independent (Slatkin, 1985a). Gene flow can be measured using both direct and indirect methods (Slatkin, 1985b, 1987). Direct methods, which use estimates of dispersal and breeding success of dispersers to infer gene flow, are biased in that they are limited in their duration and geographic extent. They also do not necessarily represent historical patterns of gene flow. Indirect methods use estimates of genetic variation, generally from biochemical procedures.

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Two methods of indirectly measuring gene flow are commonly used: Wright's statistic (F_{ST}) for estimating the standardized variation in allele frequencies among local populations (Wright, 1951) and Slatkin's rare alleles method (Slatkin, 1985b). The main problem associated with these indirect methods is that both are sensitive to variation in population structure.

The purpose of this study was to compare estimates of gene flow using direct and indirect methods for island populations of marsh rice rats (*Oryzomys palustris*), and to examine inter-island variation in allozymes. The marsh rice rat is a semi-aquatic rodent that inhabits salt marshes south of Maryland. Previous studies determined that this species inhabits more islands than any other small mammal on the Virginia barrier islands (Dueser and Porter, 1986). It has been hypothesized that the ability of the marsh rice rat to disperse across water is responsible for its ubiquity (Humphrey and Setzer, 1989).

METHODS

This study was conducted in 1989 at the Virginia Coast Reserve Long-Term Ecological Research site on the Virginia barrier islands. The barrier islands are a Holocene formation on the seaward margin of the Delmarva Peninsula. Four islands (Hog, Parramore, Revel, and Crescent) and the adjacent mainland marsh (near the town of Brownsville) were chosen due to their accessibility and proximity to each other (Figure 1). Parramore (2,197 ha in 1989) and Hog Island (1,177 ha in 1989) are barrier islands, and Revel (508 ha in 1989) is a bayside island. At the time of this study, Crescent was a small (10.0 ha) distinct island formed during the previous 20 years by sand accretion and inlet formation. Parramore and Crescent were separated by a 50 m water channel that ranged between 2-5 m deep. In October 1991, after this study was completed, a storm caused the channel separating Parramore and Crescent islands to fill with sand. The channel between Revel and Crescent is 300 m of deep water, and Hog and Parramore are separated by more than 1 km of deep water. The marsh near Brownsville is nearly equidistant from Hog and Parramore Islands. The vegetation of the four islands and mainland marsh differs slightly, but all support extensive *Spartina alterniflora* marsh, which is important rice rat habitat (McCaffrey and Dueser, 1990; Dueser and Porter, 1986)

To directly monitor inter-island movements of rice rats, determine the fate of inter-island movers, and estimate population density, trapping grids with Sherman live-traps spaced 15 m apart were laid in areas that were closest to adjacent islands. On the small island of Crescent and the narrow peninsulas of Parramore and Revel, a grid design of 6 by 20 traps was chosen because it covered the entire extent of Crescent Island and covered the width of the narrow peninsulas. On Hog and Brownsville, traps were laid in two long transects in an attempt to cover the extent of the marsh closest to the other islands. Each transect was 885 m long, and the two transects were spaced approximately 30 m apart. These transects covered approximately the same area as the island grids.

Trapping occurred May 20 through August 24, 1989. All small mammals caught were ear-tagged and examined for weight, sex, and reproductive condition, e. g., gravid, sexually active (males, testes descended; females, vagina perforate). During the first trapping session on Crescent Island, total enumeration and tagging of all *O. palustris* was attempted. Trapping continued until no untagged animals were

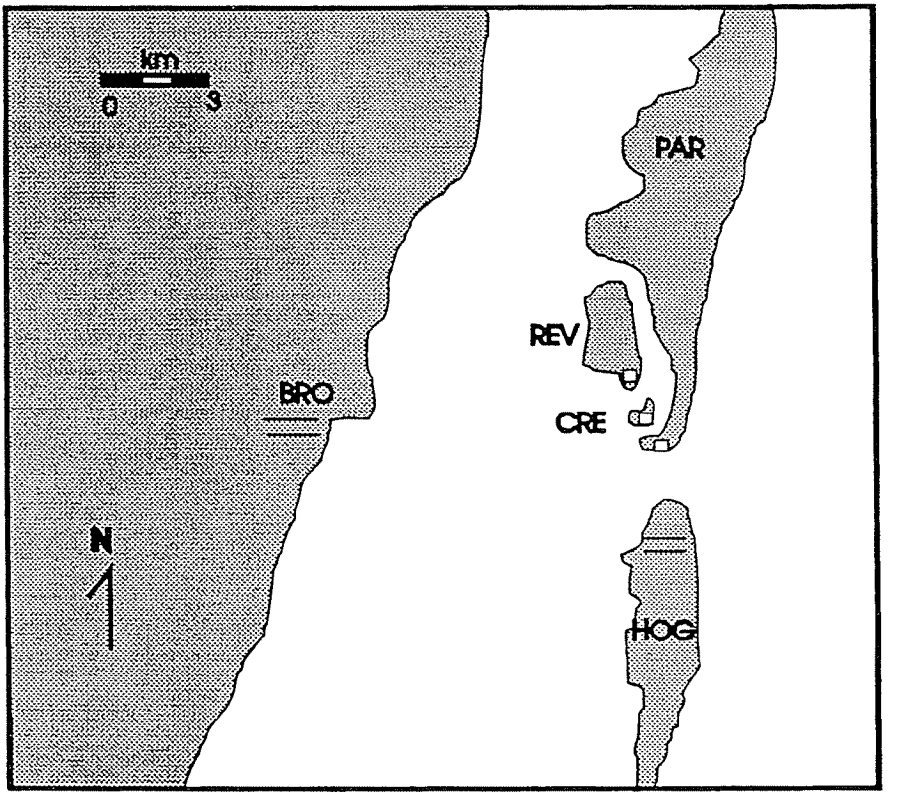

FIGURE 1. The study sites, four islands of the Virginia Barrier Island formation (PAR = Parramore, CRE = Crescent, REV = Revel, and HOG = Hog) and the mainland (BRO = Brownsville), as they appeared in 1989. The squares represent grids and the lines represent transects.

caught for three consecutive days, creating an 8-day trapping session. The individuals tagged were assumed to be residents of Crescent Island. Three of the islands (Parramore, Revel, and Crescent) and the mainland were trapped monthly (June, July, August) using the 8-day trapping session. The Hog Island site was trapped monthly using a comparable trapping session. Density was estimated using the minimum number alive method (MNA; Hilborn et al., 1976). MNA was chosen because it only considers the number of animals caught during each trapping session, allowing a comparison of the number of tagged rice rats that moved among islands to be made. Because it was difficult to differentiate between inter-island dispersers and juveniles born on the island, inter-island dispersers were defined as those animals previously tagged which moved to another island. Age was estimated using a weight proxy, with juveniles <30 g, subadults 30-50 g, and adults >50 g (Negus et al., 1961).

To indirectly estimate gene flow, allozymes were analyzed using blood samples (< 1 mL) taken from the tails of all the marsh rice rats (> 20 g) caught on the grids of Crescent, Parramore, and Revel and on the transects of Hog and Brownsville. Animals were transported to the laboratory, bled, and held overnight with food and water *ad libidum*. The next day each individual was released on the grid or transect from which it had been removed. Each animal was bled only once. Blood was stored in capillary tubes at -20°C and analyzed electrophoretically within one year.

Allozymes for 13 presumptive genetic loci were examined for 109 individuals from the five sites; 28 individuals each from Crescent and Revel, 8 individuals from Parramore, 25 rice rats from Hog and 20 from Brownsville. Standard procedures for starch-gel electrophoresis were used (Harris and Hopkinson, 1976; Murphy et al., 1990). Locus nomenclature followed McAlpine et al. (1985) for mapped human genes. The 13 allozymes were analyzed using three buffer systems. We used tris-citrate, pH 6.7 for hexokinase, Enzyme Commission (E.C.) 2.7.1.1 (HK); isocitrate dehydrogenase, E.C. 1.1.1.42 (ICD-1); glucose phosphate isomerase, E.C. 5.3.1.9 (GPI); beta hemoglobin (BHb); lactate dehydrogenase, E.C. 1.1.1.27 (LDHA); malate dehydrogenase, E.C. 1.1.1.37 (MDH-1); and purine nucleoside phosphorylase E.C 3.4.2.1 (NP). Using tris-citrate, pH 8.0, we analyzed adenosine deaminase, E.C. 3.5.4.4 (ADA), glutamic-oxaloacetic transaminase, E.C. 2.6.1.1 (GOT-1); peptidases, E.C. 3.4.13 (PEPC: L-leucyl-L-alanine as substrate; PEPD: L-phenylalanyl-L-proline as substrate); and 6-phosphoglucomutase dehydrogenase, E.C. 1.1.1.44 (6-PGD). Poulik was used for superoxide dismutase, E.C. 1.15.1.1 (SOD-1). Numerous side-by-side comparisons of electromorphs were made to assure correct assessment of relative mobilities. Electromorphs were assumed to represent alleles and were assigned unique letters, with "A" designating the most common allele.

Allozymic results were summarized and analyzed statistically using BIOSYS-1 (Swofford and Selander, 1981). Hardy-Weinberg equilibrium was tested using exact significance probabilities. Chi-square contingency tests were used to test for homogeneity of allele frequencies among populations. Gene flow (Nm) was estimated using Wright's (1951) original formula. This method was chosen over the rare alleles method (Slatkin, 1985b) because of the problems associated with detecting and scoring rare alleles in electrophoresis. A phenogram was constructed using UPGMA (Sneath and Sokal, 1973) and a matrix of Rogers' (1972) genetic distances between pairs of populations. Mantel (1967) tests were used to test the association between geographic and genetic distances.

RESULTS

Direct method of measuring gene flow

Of the 219 rice rats tagged during the summer, only 11 (5%) were observed to have made inter-island movements. All movements were of rice rats leaving Crescent Island (the small island between the two larger islands) and arriving at the study sites on Parramore and Revel. Ten rice rats from Crescent were trapped 50 m away on Parramore and one Crescent rice rat was trapped 300 m away on Revel. Averaging the number of movements between each site over the total

TABLE 1. Allelic frequencies for the three variable loci[a] in one mainland and four island populations of *Oryzomys palustris* on the Virginia Barrier Islands.

Locus	Allele	Site				
		Crescent (28)	Revel (28)	Parramore (8)	Hog (25)	Brownsville (20)
ADA	A	0.268	0.130			0.300
	B	0.554	0.574	0.714	0.500	0.450
	C	0.179	0.296	0.286	0.500	0.250
6-PGD	A	0.667	0.618	0.333	0.500	0.867
	B	0.333	0.382	0.667	0.500	0.133
SOD-1	A	0.482	0.482	0.429	0.280	0.639
	B	0.518	0.518	0.571	0.720	0.361

[a]The following loci were monomorphic: GPI, GOT-1, Hb, HK, ICD-1, LDHA, MDH-1, NP, PEPC, PEPD.

number of site pairs (16 possible movement directions), the direct estimate of gene flow was 0.75 migrants per generation for the five sites.

Three of the dispersers were juveniles (two males, one female), three were subadults (all females) and five were adults (all males). All inter-island dispersers were trapped for the duration of the study only at the sites to which they had migrated. All of the subadults and adults appeared to be sexually active, but none of the females were noticeably pregnant during the summer.

Indirect method of measuring gene flow

Three polymorphic loci (ADA, 6-PGD, and SOD-1) were identified in the five populations (Table 1). Deviations from Hardy-Weinberg expectations were observed ($P < 0.001$) at a single locus (ADA) in three of the populations (Crescent, Revel and Brownsville). The F_{IT} and F_{IS} values (measures of deviation from Hardy-Weinberg proportions) were positive for all three of the loci, indicating an excess of homozygotes. Highly significant genic differentiation was observed among the sites at all three polymorphic loci: ADA ($X^2 = 33.82$, d.f. $= 8$, $P < 0.001$), 6-PGD ($X^2 = 21.04$, d.f. $= 4$, $P < 0.001$), SOD-1 ($X^2 = 11.14$, d.f. $= 4$, $P < 0.025$), and overall heterogeneity was highly significant ($X^2 = 66.00$, d.f. $= 16$, $P < 0.001$).

The estimates of Nm (Table 2) were all greater than one, indicating that gene flow was sufficiently strong to prevent genetic drift from causing local genetic differentiation (Slatkin, 1987). To estimate the actual amount of the population immigration (m) it is necessary to make an estimate of the average effective population (N_e). There was not sufficient data to calculate N_e. However, because of a prior study (Forys, 1990) that documented the sex ratio for 15 rice rat

TABLE 2. The estimates of Nm and F_{ST} for three polymorphic loci from one mainland and four insular populations of *Oryzomys palustris*.

Locus	F_{ST} (mean)	Nm(estimated)
ADA	0.062	3.78
6-PGD	0.130	1.67
SOD-1	0.053	4.47

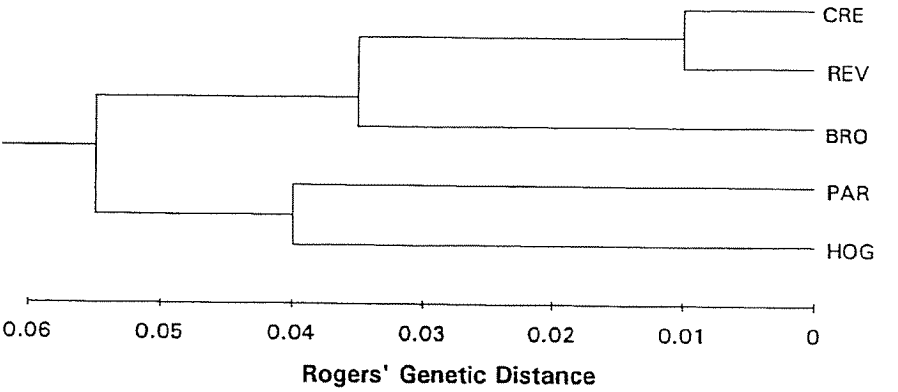

FIGURE 2. UPGMA phenogram depicting relationships among the five populations (located on: PAR = Parramore, CRE = Crescent, REV = Revel, HOG = Hog, and BRO = Brownsville) of *Oryzomys palustris* based on Rogers' genetic distance (1972).

populations to be near 1:1, the assumption was made that Ne = 2N (Lande and Barrowclough, 1987).

To calculate the average population size (N), the average density of the five sites (X = 8.78, SE = 2.83) (Forys and Dueser, 1993) was used. Extending this estimate over the area of the grids, the average population size (N) was 18.79 rats. The equation below was used to calculate the number of migrants per generation (m) using Wright's (1951) original formula with the substitution of Ne, where F_{ST} is the measure of the genetic differentiation of subpopulation.

$$m = \frac{F_{ST}}{2(N)}$$

Using this equation the estimate of m is 0.09, or 0.09 migrants per generation among the five sample sites.

Genetic distance

Rogers' (1972) genetic distance coefficients ranged from 0.014 for the Revel-Crescent comparison to 0.079 for the Parramore-Brownsville comparison. The UPGMA phenogram (Figure 2) indicated two major groupings: the adjacent islands of Crescent and Revel grouped with the mainland site (Brownsville), and the larger barrier islands of Hog and Parramore formed a separate group. The Mantel test indicated that genetic and geographic distance were not correlated for the five populations ($r = 0.064$, $P = 0.482$).

DISCUSSION

During the summer of 1989, movements of *O. palustris* across 50 m of water occurred several times (10) per generation (1 year), but dispersal across 300 m of water was recorded only once. No movement was observed between sites separated by more than 300 m. It is possible that more over-water movements may have occurred during the year, but the summer was deemed the most likely time of year for inter-island dispersal by small mammals due to the higher water temperature.

The direct estimate of gene flow (0.75) was over eight times the indirect estimate (0.09) for the same five sites. This could be due, in part, to experimental errors caused by sampling individuals for the indirect estimate of gene flow from only one part of each island. These subpopulations may not be representative of the entire island population, and the genetic composition of the subpopulations may have been affected not only by inter-island movements but by intra-island movements of individuals. However, this potential sampling bias does not completely explain the higher direct estimate. The higher direct estimate may indicate that, although individuals are successfully crossing the water barrier and surviving on islands to which they dispersed, they are not successfully breeding. This explanation is supported by the fact that the five sites were significantly different genetically despite the number of movements seen during the summer of 1989. Several studies of small mammal populations have documented the inability of immigrants to penetrate established groups (Festa-Bianchet and King, 1984; Schwartz and Armitage, 1981).

The discrepancy in the direct and indirect estimates obtained in this study may also reflect the differences in the time scales incorporated by these estimates. The barrier island complex is highly susceptible to storms (as evidenced by the storm in October 1991 that eliminated the water channel between Parramore and Crescent islands). Geologic evidence suggests that the larger barrier islands have migrated landward by both erosion on their seaward margins and accretion on their bayside (Dolan et al., 1979). It is possible that in the recent past, some of the islands included in this study were spaced farther apart and/or that some of the low-lying marshes between the islands (that may serve as stepping stones) have changed their configurations.

These changes in the configurations of the barrier islands and the effects of storms may also explain the fact that the Mantel test did not reveal a correlation between genetic and geographic distance. It is noteworthy that the greatest disparity in the geographic and genetic distances was between rice rat populations on Crescent and Parramore Islands. Despite being separated from Parramore by only 50 m of water, Crescent Island is more similar to Revel (300 m away) and the

mainland (> 7 km away) than it is to the population on Parramore. The Parramore population was most similar to that on Hog in terms of genetic distance. These genetic affinities may be the result of recolonization from different source populations following storms and island movements. For example, after the large Northeastern storm of 1988 when the southern tip of Parramore and Crescent were overwashed, Crescent may have been recolonized by individuals on Revel, while the site on Parramore was recolonized by individuals from other marshes on Parramore. More data on the geologic history of the barrier islands and a more comprehensive assessment of genetic variation among populations are needed before a complete colonization scenario can be proposed for the *O. palustris* populations on the Virginia Barrier Islands.

ACKNOWLEDGMENTS

This is a contribution of the Virginia Coast Reserve Long-Term Ecological Research Program, supported by NSF grant BSR - 8702333 to the University of Virginia. We thank R. Dueser for his support and comments on earlier drafts.

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