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I am submitting herewith a thesis written by John B. Anderson entitled "Breeding Systems and Genetic Population Structure in Philomycid Slugs (Mollusca: Pulmonata)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

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BREEDING SYSTEMS AND GENETIC POPULATION STRUCTURE IN PHILOMYCID SLUGS (MOLLUSCA: PULMONATA)

A Thesis

Presented for the

Master of Science

Degree

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John B. Anderson

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DEDICATION

This work is dedicated

to my parents, who made it all possible,

and

to Ruby and Grace, who made it all worthwhile.

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ABSTRACT

Four species of hermaphroditic terrestrial slugs of the family Philomycidae (Mollusca: Pulmonata) were collected at 13 sites in Tennessee and one site in Virginia. A total of 343 individuals were examined electrophoretically at 11 allozyme loci. These data were used to assess: (1) breeding system in all four species, (2) interdemic and interspecific systematic relationships, and (3) demic structure in Philomycus carolinianus, the most abundant species collected. Breeding system was inferred from analysis of genotypic frequencies within demes. It was concluded that all four species use outcrossing as the primary breeding system in all populations examined. The genetic identities among demes, among species and among genera were similar to values for the same comparisons in other studies and are in good agreement with systematics based on morphological characters. In the one species examined in greatest detail, Philomycus carolinanus, there was significant heterogeneity among demes. This differentiation among demes was attributed to genetic drift due to restricted gene flow. Genetic identities were not significantly correlated with geographic distance between demes. Analysis using hierarchical F-statistics demonstrated that more than half of the differentiation among demes was accounted for by differentiation at the smallest geographic scale. The Tennessee River system also appeared to form a major barrier to gene flow. It was concluded that the populations of

Philomycus carolinianus are highly fragmented and gene flow among these demes is restricted by distance and the barrier of the Tennessee River.

TABLE OF CONTENTS

CHAPTER	PAGE
1. INTRODUCTION	1
2. MATERIALS AND METHODS	6
Collections	6
Electrophoresis	9
Statistical Methods	11
3. RESULTS	12
Collections	12
Variability	12
Genotype Frequencies within Demes	18
Genetic Identities	20
Interdemic Heterogeneity in *Philomycus carolinianus*	24
4. DISCUSSION	30
Breeding System	30
Interspecific Demography	34
Interspecific Genetic Similarity	37
Geographic Variability in *Philomycus carolinianus*	37
LITERATURE CITED	42
VITA	47

LIST OF TABLES

TABLE		PAGE
1.	Areas and elevations of 14 collection sites	7
2.	Number of Philomycus carolinianus, P. venustus, Pallifera varia and Megapallifera mutablis collected at 14 collection sites	13
3.	Allele frequencies at 11 loci in 13 collections of Philomycus carolinianus	14
4.	Allele frequencies at 11 loci in Philomycus venustus, Megapallifera mutablis, and Pallifera varia	16
5.	Excess or deficiency of observed heterozygote frequencies from Hardy-Weinberg expectation at each locus for each deme of Philomycus carolinianus	19
6.	Excess or deficiency of observed heterozygote frequencies from Hardy-Weinberg expectation at each locus for each deme of Philomycus venustus	21
7.	Nei's Genetic Identity, and Rogers' Genetic Similarity between all pairs of 13 samples of Philomycus carolinianus	22
8.	Nei's Genetic Identity averaged by species	23
9.	Genetic heterogeneity among 13 collections of Philomycus carolinianus	26

TABLE PAGE

10. Nei's Genetic Identity averaged within and among groups of demes 27

11. Nei's Genetic Identity averaged within and among regions 29

12. Hierarchical F-statistics, variance components, and percent of total among deme variance 29

CHAPTER 1

INTRODUCTION

The most common breeding system in most animals and plants is outcrossing. Most flowering plants, however, are hermaphrodites (Lewis, 1942) and the frequency of self-fertilization can be very high in some species (Stebbins, 1974; Allard, et al., 1977; Willson, 1983). The frequency of hermaphroditism in animals is much lower than in plants and hermaphroditism is common only in invertebrates (for a review, see Ghiselin, 1969). The actual frequency of self-fertilization in animal hermaphrodites is known for relatively few species, but there is growing evidence that in some groups of animals, self-fertilization is more common than generally assumed (Ghiselin, 1974; Foltz et al., 1984).

Self-fertilization may offer an advantage to the individual by eliminating the necessity of finding a mate. This would allow reallocation of the energy expenditures that usually are associated with mating, such as for courtship behaviors in animals or for structures that aid in pollen dispersal in plants, to other activities (Maynard Smith, 1978). Removal of the need to find a mate also may be important to individuals when population densities are low, where it might be too costly or even impossible to find a mate. The ability to self-fertilize could provide a strong advantage in such a situation. Self-fertilization may be especially useful for an individual colonizing a new area where mates may be absent or rare.

The selfing individual is also at an advantage because its offspring carry 100% of its genes compared with an outcrossing individual whose offspring carry only 50% of its genes. This is similar to the so-called twofold advantage of parthenogenesis (Maynard Smith, 1978). If there is no difference in fecundity and no corresponding decrease in the relative fitness of offspring, then the genetic fitness of the selfing individual is effectively doubled over that of outcrossers (Maynard Smith, 1978).

Selfing, like other forms of close inbreeding, may be disadvantageous because it rapidly reduces the amount of heterozygosity that the average individual carries. This generally is believed to result in inbreeding depression, the reduction in vigor of inbred lines, through the expression of deleterious recessive traits that are not expressed in the heterozyogote (Falconer, 1981). Inbreeding depression ultimately can lead to extinction of the line. Furthermore, inbreeding may reduce the range of habitats that an individual may occupy since greater heterozygosity presumably generates a greater range of responses to environmental conditions (Dobzhansky et al., 1977). Offspring resulting from self-fertilization have less heterozygosity and so might be at a competitive disadvantage in a variable environment to offspring resulting from outcrossing. For these reasons, it generally is believed that self-fertilization is avoided where possible in natural populations (Dobzhansky et al., 1977; Hartl, 1980).

On the other hand, a reduction in variability may have certain advantages. There is no a priori reason that a monogenic genome is necessarily detrimental to the individual. If the monogenic genome that is produced by selfing is particularly well suited to a specific microniche, or if it produces high phenothypic plasticity, then it would be favored. High homozygosity in the individual precludes functional recombination during meiosis since the chromosome segments exchanged are nearly identical. Self-fertilization would then be an advantage because it prevents the breaking up of an adaptive genome by recombination (Futuyma, 1979). Preservation of a generalist genotype coupled with assured reproduction in a lone individual by self-fertilization could be optimal for individuals in certain habitats (Baker, 1965; 1972).

Even in populations where individuals outcross exclusively, factors such as dispersal and vagility can be important determinants of the genetic make-up of a population. A large, panmictic population will have uniform distribution of genotypes within the population. In contrast, in situations where the population is divided into small semi-isolated demes, there is likely to be heterogeneity among demes and less heterozygosity within single demes because of the effects of inbreeding and genetic drift. The genetic structuring of semi-isolated conspecific demes is apparently quite common within natural populations of plants and animals (Selander and Johnson, 1973; Selander and Kaufman, 1975; Ayala, 1976; Jones et al., 1980; Selander and Whittam, 1983). In some cases (see reviews

in Hedrick, et al., 1976; Koehn, 1978) it has been demonstrated that this differentiation is the result of directional processes, such as differential selection. However, it is also hypothesized that stochastic processes, like genetic drift, alone are sufficient to account for substantial differentiation among demes and this has been shown to be the case in many studies (e.g. Buri, 1956; Kidd and Cavalli-Sforza, 1974).

Theoretically, the smaller and more isolated the demes are, the more extreme the genetic differentiation due to drift will be. In animals, migration rate is the major factor in determining the amount of gene flow among demes. Therefore, all other things being equal, animals with low migration rates are likely to show higher levels of differentiation at a smaller geographic scale than more mobile organisms.

This project deals with the Philomycidae, an endemic family of North American hermaphroditic land molluscs. Philomycids are especially interesting because they are the only family of pulmonate slugs that are endemic to North America. Although philomycids are often the most common slug in eastern woodlands, they are poorly described taxonomically and relatively little is known about their biology. They are simultaneous hermaphrodites but the frequency of self-fertilization has not been thoroughly examined (McCracken and Selander, 1980). The most common species are primarily arboreal and are restricted to undisturbed woodlands. These slugs tend to aggregate in these microhabitats with several

individuals often found together on single trees and few or none in the areas between suitable trees. Since slugs have rather low vagility, population structuring may be more apparent than in more mobile organisms.

This project had two goals. These were: (1) to examine the frequency of self-fertilization in natural populations of philomycid slugs, and (2) to analyze the geographic distribution of population genetic structure in several local populations of these animals in Tennessee.

CHAPTER 2

MATERIALS AND METHODS

Collections

Slugs were collected from 13 localities in Tennessee and 1 in Virginia. All collections were made between April 1983 and July 1985. The collection localities include 4 sites on Chilhowee Mountain, Blount County, Tennessee; 3 sites on Clinch Mountain, Grainger County, Tennessee; 3 sites on the University of Tennessee Woodlot, Knox County, Tennessee; 3 sites near Powell, Knox County, Tennessee; and 1 site near Mountain Lake Biological Station, Pembroke, Virginia. All sites were in relatively undisturbed stands of mixed deciduous forest, except for one site (Powell 1) which was in a wooded lawn adjacent to a large woodlot. Collections were made in daylight by searching for slugs on the outer surface or under loose bark of standing or fallen dead hardwood trees. Collection success was greatest in the early morning hours on warm rainy days in the spring and autumn. Each site was searched intensively and all slugs found were collected. Each sample was taken from as small an area as was possible. The size of collection sites ranged from approximately .001 to .8 hectare (Table 1).

The locations of the Tennessee collection sites were chosen to give a hierarchical arrangement (Figure 1). Individuals at a collection site were assumed to be potentially interbreeding and therefore to constitute a local population or deme. The

Table 1. Areas and elevations of 14 collection sites.

Locality	Area (ha.)	Elevation (M)
Chilhowee Mt. 3 (CH3)	.5	396
Chilhowee Mt. 4 (CH4)	.4	396
Chilhowee Mt. 5 (CH5)	.6	396
Chilhowee Mt. 8 (CH8)	.8	396
Clinch Mt. 1 (CM1)	.4	575
Clinch Mt. 2 (CM2)	.3	670
Clinch Mt. 3 (CM3)	.1	670
UT Woodlot 1 (UT1)	.01	305
UT Woodlot 2 (UT2)	.2	305
UT Woodlot 3 (UT3)	.01	300
Powell 1 (PO1)	.005	335
Powell 2 (PO2)	.3	365
Powell 3 (PO3)	.001	365
Mountain Lake, Va. (MLBS)	.2	1170

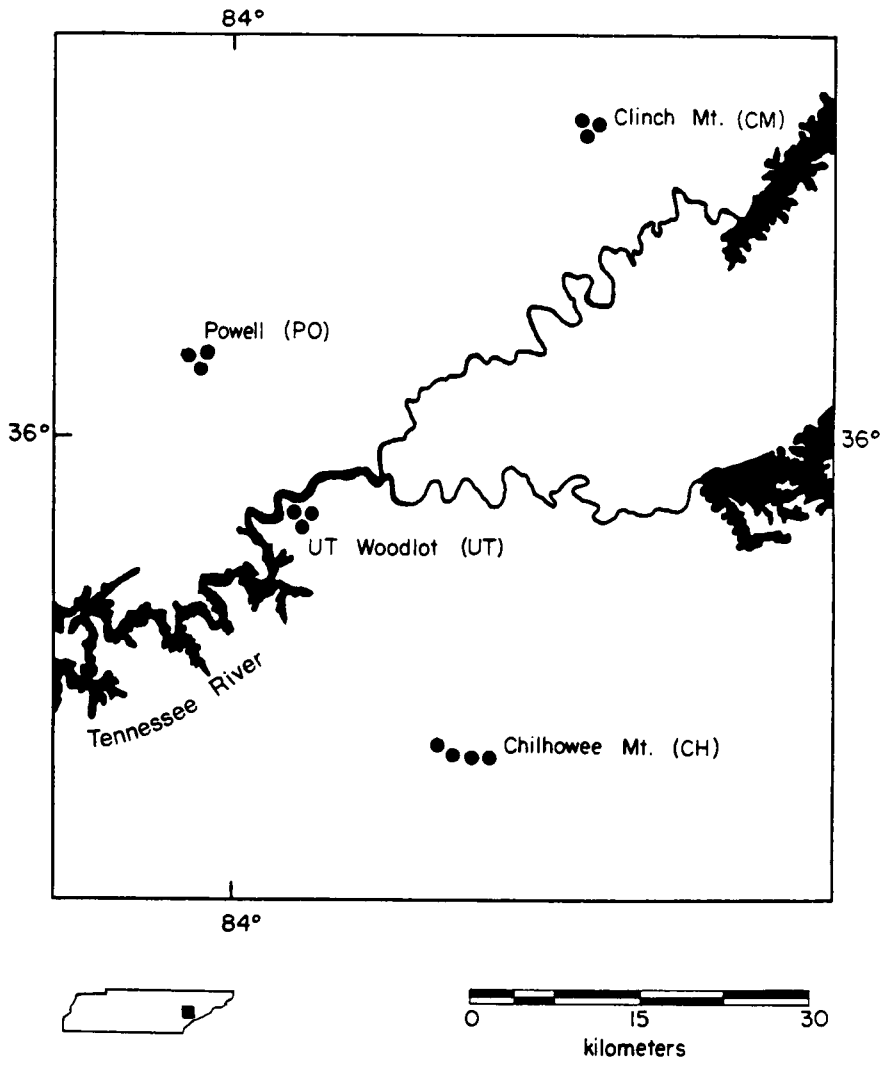


Figure 1. Map of Tennessee study area. Closed circles are individual collection sites.

sites in each major area (e.g. Chilhowee Mt.) together constitute a geographic group of demes. The demes are further grouped into geographic regions north and south of the Tennessee River system. This arrangement was chosen to allow analysis of variability at four geographic scales: (1) within individual sites or demes, (2) within geographic groups consisting of demes less than 3 Km apart, (3) among groups of demes within regions, and (4) the total study area (Figure 1). For example, each of the three sites (UT1, UT2, UT3) at the UT Woodlot represents a deme, the three demes together represent a group of demes (UT), the UT Woodlot sites together with the four Chilhowee Mountain sites represent a region (SOUTH), and finally all 13 sites represent the total study area (TOTAL).

Specimens were transported alive to the laboratory and then immediately placed in an ultra-cold freezer at -70°C until preparation for electrophoresis. Animals were numbered in order of collection and identified to species by morphological characters, with a representative of each species preserved for voucher. Dr. Lee Fairbanks of Pennsylvania State University independently confirmed identifications.

Electrophoresis

Methods of tissue preparation followed McCracken and Selander (1980). For small individuals (< 1g), the whole animal was used. For larger individuals (up to 4g), about 1g of tissue was used

and the rest refrozen. In most cases, when the whole animal was not used, the tissue used included both foot muscle and digestive tissue. All samples were kept on ice at all times after thawing. Each tissue sample was weighed and the tissue was minced in a spot plate with sharp scissors. Each sample was then placed in a centrifuge tube, mixed with a pH 6.8 grinding buffer (Selander et al., 1971) at a 1:1 wt/vol ratio, placed on ice, and allowed to stand for 30 minutes for extraction of the soluble proteins. The tubes were then centrifuged at 2°C and 13,000 r.p.m. for 40 minutes. The supernatant liquid was removed with a pipette and placed in a labelled tube for storage in the ultra-cold freezer.

Horizontal starch gel electrophoresis followed the methods of Harris and Hopkins (1978) and Selander et al. (1971). A survey of 34 enzyme stains revealed 11 loci that could be scored reliably: 6-phosphogluconate dehydrogenase (6-PGD), superoxidase dismutase (SOD), mannose phosphate isomerase (MPI), glutamate oxalate transaminase (GOT), leucine aminopeptidase 1 and 2 (LAP1 and LAP2), exterase 2 and 3 (EST2 and EST3), phosphoglucomutase (PGM), glucose-6-phosphate dehydrogenase (G-6-PD), and phosphoglucose isomerase (PGI). The preferred buffer systems (Selander et al., 1971) were: Lithium hydroxide (EST, LAP and GOT); continuous Tris Citrate pH 8.0 (6-PGD, SOD and MPI); discontinuous Citrate (Poulik) (PGI); and TEB gel buffer with Borate tray buffer (PGM and G-6-D). Alleles at each polymorphic locus were designated alphabetically in order of decreasing electromobility.

Statistical Methods

Observed allele and genotype frequencies were determined by direct count. Expected genotype frequencies and expected heterozygosity were calculated from allele frequencies, with correction for small sample size (Levene, 1949). Conformance to expectations of Hardy-Weinberg equilibrium was tested by the G-test of goodness of fit (Sokal and Rohlf, 1981), with genotypes pooled as either heterozygotes or homozygotes. Overall patterns of heterozygote excess or deficiency were tested by the sign test (Siegel, 1956). Genetic heterogeneity among demes was tested with the G-test for heterogeneity (Sokal and Rohlf, 1981), with rare alleles pooled. F-statistics for each locus were obtained by Wright's (1978) method of hierarchical analysis. Genetic similarity among demes was estimated by Nei's (1978) unbiased identity (I) and by Rogers (1972) similarity (S). Nei's Identity was averaged by genus and species and at various hierarchical scales reflecting the geographical locations of the collection sites. Cluster analysis (Sneath and Sokal, 1973) was performed using the unweighted pair-group method (UPGMA) based on Nei's I. The BIOSYS-I computer program (Swofford and Selander, 1981) was used to perform the genetic analyses.

CHAPTER 3

RESULTS

Collections

A total of 343 slugs of four species were collected and examined electrophoretically. The number of individuals of each species collected at each site is given in Table 2. Collections of Philomycus carolinianus ranged in size from 7 to 39 individuals with a mean of 19.3. These samples were used both for examination of breeding system and for examination of microgeographic population structuring. Five collections of Philomycus venustus ranging in size from 3 to 23 individuals, two collections of Megapallifera mutablis, and one collection of Pallifera varia were also obtained. The small number of samples and sometimes small sample sizes obtained for these later three species did not allow analysis of geographic variation; however, assessment of their breeding systems was possible.

Variability

Allele frequencies for 11 loci are given for Philomycus carolinianus in Table 3. Allele frequencies for the other three species are in Table 4. For a given locus, use of the same alphabetic allelic designation among species indicates that there were no detectable differences in electromobility and the alleles are assumed to be identical. Six of the 11 loci are robustly

Table 2. Numbers of *Philomycus carolinianus*, *P. venustus*, *Pallifera varia* and *Megapallifera mutablis* collected at 14 collection sites.

Site	Species			
	P. carolinianus	*P. venustus*	*M. mutablis*	*P. varia*
Chilhowee Mt. 3	39	3	0	0
Chilhowee Mt. 4	18	0	0	0
Chilhowee Mt. 5	34	0	0	0
Chilhowee Mt. 8	15	0	0	0
Clinch Mt. 1	21	4	0	0
Clinch Mt. 2	15	23	0	0
Clinch Mt. 3	17	0	0	0
U.T. Woodlot 1	14	0	0	0
U.T. Woodlot 2	16	0	0	0
U.T. Woodlot 3	13	0	0	0
Powell 1	22	0	31	0
Powell 2	20	17	0	0
Powell 3	7	8	5	0
MLBS	0	0	0	19
Total	251	37	36	19

Table 3. Allele frequencies at 11 loci in 13 collections of Philomycus carolinianus.

Locus	Allele	Collection												
		CH3	CH4	CH5	CH8	CM1	CM2	CM3	UT1	UT2	UT3	PO1	PO2	PO3
6PGD	C	1.0	1.0	1.0	1.0	0.976	0.941	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	D	0.0	0.0	0.0	0.0	0.024	0.067	0.059	0.0	0.0	0.0	0.0	0.0	0.0
SOD	B	0.0	0.0	0.015	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C	1.0	1.0	0.985	1.0	1.0	1.0	1.0	0.929	0.969	0.923	1.0	1.0	0.929
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.071	0.031	0.077	0.0	0.0	0.071
GOT	C	1.0	0.01	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MPI	A	0.179	0.028	0.074	0.033	0.071	0.067	0.088	0.036	0.031	0.0	0.0	0.025	0.0
	B	0.577	0.861	0.779	0.833	0.881	0.767	0.794	0.714	0.781	0.731	1.0	0.90	0.714
	C	0.244	0.111	0.147	0.133	0.048	0.167	0.118	0.250	0.188	0.269	0.0	0.075	0.286
LAP1	A	0.031	0.0	0.044	0.0	0.0	0.033	0.0	0.0	0.0	0.0	0.0	0.025	0.143
	B	0.625	0.611	0.588	0.533	0.5	0.867	0.676	0.750	0.594	0.615	0.864	0.425	0.857
	C	0.344	0.389	0.368	0.467	0.476	0.10	0.324	0.214	0.406	0.385	0.136	0.550	0.0
	D	0.0	0.0	0.0	0.0	0.024	0.0	0.0	0/036	0.0	0.0	0.0	0.0	0.0
LAP2	B	0.026	0.167	0.015	0.1	0.0	0.0	0.0	0.0	0.188	0.0	0.0	0.0	0.0
	C	0.962	0.806	0.956	0.733	1.0	0.967	0.893	0.813	0.885	1.0	1.0	1.0	1.0
	D	0.013	0.028	0.029	0.167	0.0	0.033	0.0	0.107	0.0	0.0	0.0	0.0	0.0
EST2	A	0.179	0.0	0.158	0.214	0.133	0.036	0.357	0.0	0.0	0.0	0.0	0.575	0.3
	B	0.256	0.353	0.421	0.179	0.567	0.5	0.286	0.591	0.385	0.269	0.614	0.325	0.4
	C	0.564	0.647	0.421	0.607	0.3	0.464	0.357	0.409	0.615	0.731	0.386	0.1	0.3
EST3	A	0.282	0.278	0.25	0.167	0.262	0.0	0.0	0.393	0.281	0.115	0.0	0.025	0.0
	B	0.590	0.639	0.382	0.333	0.524	0.5	0.529	0.214	0.094	0.346	0.977	0.95	0.786
	C	0.026	0.083	0.191	0.367	0.214	0.5	0.471	0.286	0.5	0.5	0.023	0.025	0.214
	D	0.103	0.0	0.176	0.133	0.0	0.0	0.0	0.107	0.125	0.038	0.0	0.0	0.0

Table 3 (continued)

Locus	Allele	Collection												
		CH3	CH4	CH5	CH8	CM1	CM2	CM3	UT1	UT2	UT3	PO1	PO2	PO3
PGM	A	0.0	0.0	0.015	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.071
	B	0.321	0.528	0.515	0.433	0.643	0.733	0.706	0.643	0.357	0.462	0.909	0.825	0.714
	C	0.679	0.472	0.471	0.567	0.357	0.267	0.294	0.357	0.643	0.538	0.091	0.175	0.214
G6PD	B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.095	0.0	0.0
	C	0.974	0.917	0.926	0.933	0.974	0.967	0.882	0.964	1.0	1.0	0.857	0.925	1.0
	D	0.026	0.083	0.074	0.067	0.026	0.033	0.118	0.036	0.0	0.0	0.048	0.075	0.0
PGI	A	0.0	0.0	0.0	0.0	0.0	0.0	0.029	0.0	0.0	0.0	0.0	0.0	0.0
	B	0.0	0.0	0.0	0.0	0.0	0.033	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.987	1.0	1.0	1.0	1.0	0.933	0.971	1.0	1.0	1.0	1.0	1.0	1.0
	D	0.013	0.0	0.0	0.0	0.0	0.033	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 4. Allele frequencies at 11 loci in Philomycus venustus, Megapallifera mutablis, and Pallifera varia.

		P. venustus					M. mutablis		P. varia
Locus	Allele	CH3	CM1	CM2	PO2	PO3	PO1	PO2	MLBS
6PGD	C	1.0	0.875	1.0	1.0	1.0	0.0	0.0	0.0
	D	0.0	0.125	0.0	0.0	0.0	1.0	1.0	1.0
SOD	C	0.0	0.375	0.022	0.0	0.0	0.0	0.0	0.0
	D	1.0	0.625	0.978	1.0	1.0	1.0	1.0	1.0
GOT	B	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0
	C	1.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0
MPI	A	0.333	0.0	0.022	0.0	0.0	0.0	0.0	0.132
	B	0.667	1.0	0.543	0.294	0.313	0.0	0.0	0.737
	C	0.0	0.0	0.435	0.706	0.688	1.0	1.0	0.132
LAP1	A	0.0	0.125	0.043	0.059	0.0	0.0	0.0	0.0
	B	0.0	0.0	0.022	0.0	0.0	0.016	0.1	0.0
	C	1.0	0.875	0.935	0.941	1.0	0.919	0.9	0.868
	D	0.0	0.0	0.0	0.0	0.0	0.065	0.0	0.132
LAP2	B	0.167	0.25	0.674	0.0	0.0	0.0	0.0	0.0
	C	0.833	0.5	0.326	1.0	1.0	0.0	0.0	0.0
	D	0.0	0.25	0.0	0.0	0.0	0.968	1.0	0.0
	E	0.0	0.0	0.0	0.0	0.0	0.032	0.0	1.0
EST2	A	0.0	0.0	0.0	0.063	0.0	0.0	0.0	0.0
	B	0.167	0.250	0.087	0.281	0.125	0.0	0.0	0.0
	C	0.833	0.75	0.913	0.656	0.875	0.0	0.0	1.0
	E	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0

Table 4 (continued)

Locus	Allele	P. venustus					M. mutablis		P. varia
		CH3	CM1	CM2	PO2	PO3	PO1	PO2	MLBS
EST3	A	0.0	0.25	0.0	0.0	0.0	0.0	0.0	0.0
	B	0.5	0.125	0.022	0.029	0.125	0.0	0.0	0.0
	C	0.5	0.625	0.978	0.971	0.875	0.0	0.0	0.875
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.125
	E	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0
PGM	B	1.0	0.625	1.0	1.0	0.667	0.0	0.0	0.026
	C	0.0	0.375	0.0	0.0	0.333	0.948	1.0	0.974
	D	0.0	0.0	0.0	0.0	0.0	0.052	0.0	0.0
G6PD	B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.026
	C	1.0	1.0	0.935	1.0	1.0	0.0	0.0	0.974
	D	0.0	0.0	0.065	0.0	0.0	1.0	1.0	0.0
PGI	B	0.667	1.0	1.0	1.0	1.0	0.0	0.0	1.0
	C	0.333	0.0	0.0	0.0	0.0	1.0	1.0	0.0

polymorphic with three or more alleles in Philomycus carolinianus. Philomycus venustus shows similar levels of variability with five robustly polymorphic loci. Both Megapallifera mutablis and Pallifera varia show low variability at all loci. The observed levels of variability are typical of those found in other populations of terrestrial molluscs (Nevo, 1978; Selander and Ochman, 1983).

Genotype Frequencies within Demes

Genotype frequencies in all demes of Philomycus carolinianus are not demonstrably different from Hardy-Weinberg expectation at any locus (Table 5). Although these individual heterozygote deviations are nonsignificant, biologically important trends toward heterozygote excess or deficiency may be revealed when deviations are considered together. A consistent pattern of deviation at all loci in a deme would suggest that some factor (e.g. inbreeding, Wahlund effect, assortative mating) operating in that deme is systematically affecting genotype frequencies at all loci. Similarly, a consistent pattern of deviation at a particular locus in all demes would suggest some factor (e.g. selection, null alleles) related to the locus. This analysis (Table 5) shows no significant patterns of deviations over all loci examined within single demes but there are significantly more heterozygote deficiencies for the EST2 and PGM loci across demes than would be expected by chance.

With the exceptions of LAP2 in CM1 and PGM in PO3, genotype frequencies in all demes of Philomycus venustus are not demonstrably

Table 5. Excess (+) or deficiency (-) of observed heterozygote frequencies from Hardy-Weinberg expectation at each locus for each deme of *Philomycus carolinianus*.

	Locus												
Deme	6PGD	SOD	MPI	LAP2	LAP2	EST2	EST3	PGM	G6PD	PGI	+	-	p
CH3			-	+	+	-	+	-	+		4	3	.5
CH4			-	-	-	-	-	-	+	+	2	6	.15
CH5			-	-	+	-	-	-	+		2	5	.23
CH8			+	-	-	-		-	+		2	4	.34
CM1			+	-		-	-	-			1	4	.19
CM2	+		+	-		-	+	-		+	4	3	.5
CM3	+		-	+		+	-	-	+		4	3	.5
UT1		+	-	+	-	-	-	-			2	5	.23
UT2			-	+	-	-	-	-			1	5	.11
UT3		+	-	+	+	-	-	-			3	4	.5
PO1				+	+	+		+	-		3	1	
PO2			+	+		-	+	-	+		4	2	.34
PO3			-	+		-	-	+			2	3	.5
Total:													
+	2	2	4	8	4	2	3	2	6	2			
-	0	0	8	5	4	11	8	11	1	0			
p			.19	.29	.64	.01*	.11	.01*	.06				

Overall Total: + 34, - 48; p = .0764

*p < .05

Individual deviations were tested for statistical significance by the G-test (Sokal and Rohlf, 1981). No deme differed significantly from Hardy-Weinberg expectation. Overall patterns of deviation were tested by the Sign Test (Siegel, 1956). EST2 and PGM show significant net patterns of heterozygote deficiency.

different from Hardy-Weinberg expectation at any locus. The two loci not in Hardy-Weinberg proportions show significant ($p < .05$) observed heterozygote deficiencies (Table 6). Analysis for patterns of nonsignificant heterozygote deviation (Table 6) show no significant patterns across loci for each deme or across demes for each locus. Heterozygote deviations for Megapallifera mutablis and Pallifera varia are nonsignificant except for EST3 in Pallifera varia. This locus shows a significant ($p < .025$) deficiency of heterozygotes. Because of the small number of demes sampled for these species, no analyses of patterns of deviation were possible.

Genetic Identities

Nei's unbiased Genetic Identity Coefficient and Rogers' Similarity Coefficient were calculated for each pair of demes of all species. Table 7 gives the between deme identity and similarity indices for 13 demes of Philomycus carolinianus. Although both Nei's and Robers' coefficients were calculated for all collections, only Nei's Identity is used for all subsequent analyses. Rogers' Similarity Coefficient has been used in other studies and is included here to allow comparison with the results of these studies. Averaging genetic identity coefficients by species (Table 8) shows within species averages of 0.967 for the 13 collections of Philomycus carolinianus, 0.945 for the five collections of Philomycus venustus, and 1.0 for the two collections of Megapallifera mutablis. These values are typical for conspecific demes (Avise, 1974). Average coefficients between genera were 0.144 for the demes of Philomycus compared to the demes of

Table 6. Excess (+) or deficiency (-) of observed heterozygote frequencies from Hardy-Weinberg expectation at each locus for each deme of *Philomycus venustus*.

	Locus												
Deme	6PGD	SOD	MPI	LAP1	LAP2	EST2	EST3	PGM	G6PD	PGI	+	-	p
CH3			+	+						+	2	1	
CM1	-				-*	+	-	-		+	2	4	.34
CM2			-	+	-	+			+		3	2	.50
PO2			-	+		-					1	2	
PO3			-			+	+	-*			2	2	
Total:													
+	0	0	1	3	0	3	1	0	1	2			
-	1	0	3	0	2	1	2	2	0	0			

*$p < .05$

Table 7. Nei's Genetic Identity (below diagonal), and Rogers' Genetic Similarity (above diagonal) between all pairs of 13 samples of Philomycus carolinianus.*

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Chilhowee Mt. 3	*****	0.913	0.923	0.902	0.887	0.856	0.872	0.873	0.896	0.898	0.819	0.833	0.858
2 Chilhowee Mt. 4	0.987	*****	0.933	0.923	0.912	0.870	0.883	0.865	0.915	0.911	0.855	0.859	0.851
3 Chilhowee Mt. 5	0.989	0.993	*****	0.927	0.932	0.898	0.913	0.919	0.910	0.913	0.851	0.865	0.877
4 Chilhowee Mt. 8	0.982	0.991	0.994	*****	0.893	0.858	0.884	0.877	0.922	0.926	0.801	0.840	0.828
5 Clinch Mt. 1	0.970	0.988	0.997	0.978	*****	0.897	0.911	0.906	0.871	0.876	0.876	0.898	0.880
6 Clinch Mt. 2	0.951	0.971	0.979	0.965	0.978	*****	0.930	0.907	0.858	0.878	0.886	0.837	0.904
7 Clinch Mt. 3	0.961	0.973	0.987	0.981	0.988	0.994	*****	0.878	0.853	0.877	0.861	0.886	0.897
8 UT Woodlot 1	0.966	0.979	0.995	0.976	0.987	0.988	0.973	*****	0.894	0.908	0.856	0.816	0.885
9 UT Woodlot 2	0.968	0.975	0.986	0.997	0.967	0.961	0.958	0.985	*****	0.932	0.787	0.791	0.823
10 UT Woodlot 3	0.978	0.986	0.989	1.000	0.973	0.981	0.978	0.985	0.999	*****	0.801	0.805	0.861
11 Powell 1	0.923	0.962	0.950	0.917	0.965	0.972	0.962	0.946	0.891	0.921	*****	0.890	0.886
12 Powell 2	0.929	0.945	0.950	0.930	0.970	0.932	0.971	0.916	0.880	0.908	0.957	*****	0.876
13 Powell 3	0.961	0.968	0.975	0.950	0.974	0.996	0.993	0.972	0.928	0.958	0.986	0.969	*****

*Statistics based on 11 loci.

Table 8. Nei's Genetic Identity averaged by species (with ranges in parentheses).

Species	# of Demes	PC	PV	Meg	Pal
P. carolinianus (PC)	13	.967 (.88-1.0)			
P. venustus (PV)	5	.775 (.64-.92)	.945 (.91-.99)		
M. mutablis (Meg)	2	.105 (.03-.15)	.247 (.23-.29)	1.00 (1.0-1.0)	
Pallifera varia (Pal)	1	.539 (.43-.63)	.715 (.67-.79)	.375 (.37-.38)	*

*Only one deme of this species was sampled.

Megapallifera, 0.588 for Philomycus compared to Pallifera, and 0.375 for Megapallifera compared with Pallifera, with a mean of 0.369 among the three genera. The only comparison of species within a genus is Philomycus carolinianus vs. Philomycus venustus. These comparisons have an average value of 0.775 which is typical of identity values between congeneric species (Avise, 1974). The Unweighted Pair Group Method (Sneath and Sokal, 1973) was used to produce a dendrogram illustrating presumptive phylogenetic relationships among demes of all four species, based on Nei's genetic identity coefficient (Figure 2).

Interdemic Heterogeneity in Philomycus carolinianus

The 13 demes of Philomycus carolinianus were examined for differences in allele frequency among demes. There is significant heterogeneity of allele frequencies among demes at most loci (Table 9). All the robustly polymorphic loci examined show high heterogeneity. The existence of interdeme heterogeneity would be less detectable in weakly polymorphic loci since they contain less information.

Linear regression of genetic identity on the geographic distance between demes shows no significant relationship ($r = .003$; $p > .91$). However, averaging identity coefficients by geographic group (Table 10) reveals that, in most cases, demes within a group are on average more similar to each other than to demes in other groups. This difference is highly significant ($p < .01$,

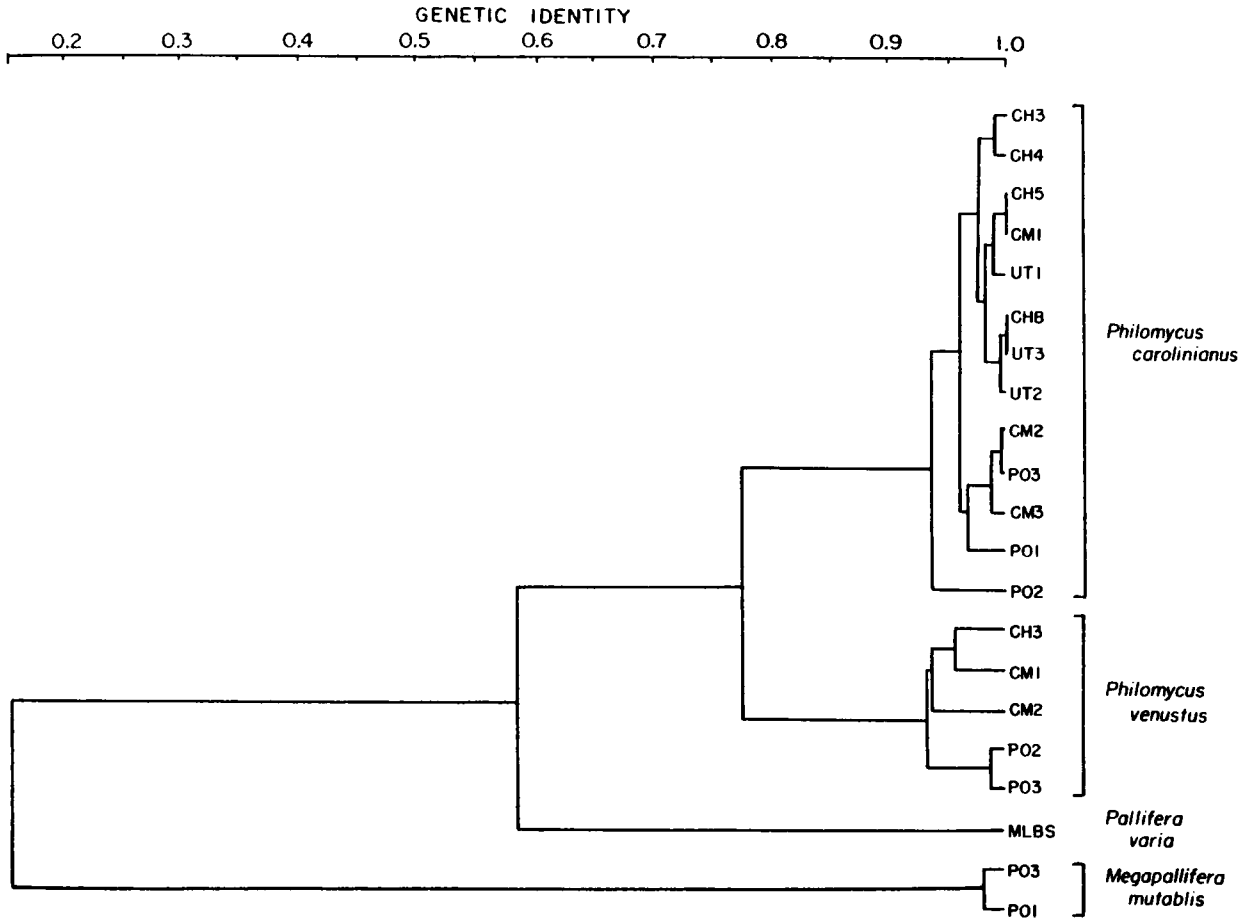


Figure 2. Dendrogram of genetic relationships among all collections, based on Nei's Genetic Identity Coefficient.

Table 9. Genetic heterogeneity among 13 collections of *Philomycus carolinianus*.

Locus	G-het*	df	p
6-PGD	16.68	12	>.1
SOD	18.61	12	>.1
MPI	51.27	12	<.001
LAP1	37.88	12	<.001
LAP2	50.84	12	<.001
EST2	52.50	12	<.001
EST3	142.39	12	<.001
PGM	74.80	12	<.001
G-6-PD	18.43	12	>.1
PGI	12.20	12	>.5

*Rare alleles pooled

Table 10. Nei's Genetic Identity averaged within and among groups of demes (with ranges in parentheses).

Group	# of Demes	CH	UT	CM	PO
Chilhowee Mt. (CM)	4	.989 (.982-.994)			
UT Woodlot (UT)	3	.983 (.966-1.00)	.990 (.985-.999)		
Clinch Mt. (CM)	3	.975 (.951-.997)	.974 (.958-.988)	.987 (.978-.995)	
Powell (PO)	3	.947 (.917-.975	.925 (.880-.972)	.971 (.932-.996)	.970 (.957-.986)

U-Test, Siegel, 1956). Averaging identity coefficients by region north and south of the Tennessee River system (Table 11) also reveals that demes are significantly more similar to other demes on the same side of the river than to demes on the opposite side of the river ($p < .001$, U-Test, Siegel, 1956).

Hierarchical F-statistics (Wright, 1978) were used to partition the overall genetic variance among demes at various hierarchical levels (Table 12). The variance component (D_{ij}, that portion of the variance accounted for by the variation among the subdivision i, within the higher level subdivision, j) is related to the F-statistics (F_{ij}, the correlation between uniting random gametes within i, relative to gametes of j) by $F_{ij} = D_{ij}/H_t$, where H_t is the total limiting variance (total diversity). This variance component is equivalent to Nei's genetic diversity (Nei, 1975) and is additive so that $D_{dg} + D_{gt} = D_{dt}$, $D_{dr} + D_{rt} = D_{dt}$ and $D_{dg} + D_{gr} + D_{rt} = D_{dt}$ (d=deme, g=group, r=region, t=total). The F_{st} value is the variance among demes and the remaining variance ($1 - F_{st}$) is the variance within demes. This analysis shows that about 91% of the variance is accounted for by genetic variability within demes. The remaining 9% is due to differentiation among demes ($D_{dt}/H_t = F_{dt} = 0.092$). Of the among deme variance, 53.4% (calculated as $D_{dg}/D_{dt} \times 100$) is due to differentiation of demes within geographic groups, 24.5% ($D_{gr}/D_{dt} \times 100$) of the variance is due to groups within regions, and 22.1% ($D_{rt}/D_{dt} \times 100$) is due to differences between regions.

Table 11. Nei's Genetic Identity averaged within and among regions, with ranges in parenthesis.

Region	# of demes	South	North
South	7	.986 (.966-1.0)	
North	6	.956 (.880-.997)	.974 (.932-.996)

Table 12. Hierarchical F-statistics, variance components, and percent of total among deme variance (calculated as (D_{ij}/D_{dt}) x 100)).

Comparison i	Comparison j	Variance Component(D_{ij})	F_{ij}	% of D_{dt}
deme	- group	.14045	.051	53.4
deme	- region	.20497	.073	77.9
deme	- total	.26296	.092	100.0
group	- region	.06451	.023	24.5
group	- total	.12250	.043	46.6
region	- total	.05799	.020	22.1

Total limiting variance (H_t) = 2.858

CHAPTER 4

DISCUSSION

Breeding System

Several families of terrestrial molluscs have been examined in other studies (Selander and Kaufman, 1975; McCracken and Selander, 1980; Foltz et al., 1982; 1984) and a variety of breeding systems have been found in closely related species. McCracken and Selander (1980) found that six of 14 species of terrestrial slugs from North America examined employed either facultative or obligatory self-fertilization as the normal breeding system. Foltz et al. (1984) found both outcrossing and selfing species of the families Limacidae and Arionidae in native habitats in Europe. Furthermore, studies of introduced populations of the same species in North America show that the breeding system apparently has not been modified in the process of colonization (Foltz et al., 1982; 1984).

The 13 collections of Philomycus carolinianus examined in the present study did not differ demonstrably from Hardy-Weinberg equilibrium at any locus examined. It can be inferred from this that the individuals in these demes are outcrossing most, if not all, of the time. The slight but nonsignificant trend toward heterozygote deficiency that was observed (Table 5, page 19) may result from sampling error or from a small amount of inbreeding that is likely in a finite population. The significant trend toward heterozygote deficiency observed at the EST2 and PGM loci

(Table 5, page 19) may be explained in ways unrelated to breeding system. There are at least three possible explanations for the consistent deficiencies of heterozygotes at these two loci. First, negative heterosis, or selection against heterozygotes, would cause a real deficiency of heterozygotes. However, whether such selection is actually occurring in these loci cannot be determined from these data. Second, the presence of one or more null alleles could result in a perceived deficiency of heterozygotes. Null alleles occur in many enzymes and apparently are especially common in esterases (Oxford, 1973; Giles, 1984). Esterase stains also are non-specific and often show complex patterns of variability resulting in problematic interpretation (McCracken and Wilkinson, in press). It is possible that the occurrence of null alleles is responsible for the patterns of deficiency observed. The third possible cause is the Wahlund effect (Hartl, 1980), which is the result of treating a sample of organisms as an interbreeding unit when, in fact, they come from two or more demes. If those demes have different allele frequencies at a particular locus then Hardy-Weinberg theory will predict more heterozygotes than there actually should be. The Wahlund effect is also a likely explanation for the observed deficiencies in these two loci. In this study, the collections were not made from an area of specific size. Instead, the search was continued in an increasing area until an acceptable number of animals was found. This may have resulted in collections from areas that were, in fact, larger than the normal range of interbreeding individuals.

If the heterozygote deficiencies observed were caused by selfing or any other type of nonrandom mating such as preferential mate selection, then all loci in a deme should show the same pattern of deviation. Since this was not the case, and genotype frequencies at most loci show no significant deviation from Hardy-Weinberg expectations, the observed results are not consistent with expectations of selfing. Thus, it can be concluded that the individuals in these demes are outcrossing most of the time.

Except for one locus in each of two demes, the five demes of Philomycus venustus also are in Hardy-Weinberg equilibrium at all loci examined (Table 6, page 21). Again, single locus deviation does not suggest that self-fertilization is a common mode of breeding in these demes, and it may be inferred that the individuals in these demes are outcrossing most of the time. The observed deficiencies at single loci also could have resulted from any of the causes considered above.

Both demes of Megapallifera mutablis and the single deme of Pallifera varia examined showed much less variability than either species of Philomycus (Tables 3 and 4, pages 14 and 16). Fewer of the loci examined were polymorphic and there were fewer alleles in those that were polymorphic. However, all polymorphic loci were close to Hardy-Weinberg equilibrium except for one locus (EST3) which showed significant heterozygote deficiency in the single collection of Pallifera varia. Long term selfing can result in reduced variability leading to monogenic strains. Existence of

such strains in populations of a species has been interpreted as evidence of exclusive or frequent self-fertilization (McCracken and Selander, 1980). However, the low variability observed in Megapallifera mutablis and Pallifera varia may be explained by other phenomena, such as population bottlenecks (e.g. Bonnell and Selander, 1974), which also can reduce the amount of variability in a population. The lack of heterozygote deficiency at most loci indicates that these two species probably are not regularly self-fertilizing.

This study has shown that the populations of the four species examined all apparently use outcrossing as their primary breeding system. This result does not necessarily apply to other populations of these species or even to these populations at all times. For example, even after several generations of self-fertilization, a single generation of random outcrossing would restore genotype frequencies to Hardy-Weinberg equilibrium.

The fact that these are hermaphrodites yet do not regularly use the potential to self-fertilize may be explained in terms of other possible advantages of hermaphroditism (Ghiselin, 1974; Maynard Smith, 1978). In a population made up of simultaneous hermaphrodites, every encounter between individuals is an encounter between potential mates. The sex ratio is always 1:1 so the effective population size is maximized. Given certain conditions, the optimal allocation of reproductive resources is to give equal energy to male and female functions (Maynard Smith, 1978). Any or all of these

considerations may account for the occurrence of hermaphroditic animals independently of breeding system.

The fact that all four of these endemic species appear to be primarily outcrossing agrees with the hypothesis that endemics are less likely to use self-fertilization than are colonizers (Foltz et al., 1984). However, at present the data on the frequency of self-fertilization in colonizers and endemics are ambiguous and any conclusion about the role of breeding system in colonizing ability cannot be made from this type of data alone. Further study on the roles of genetic variability and phenotypic plasticity as ecological strategies is needed to resolve this issue.

Interspecific Demography

Since no attempt was made in this study to selectively collect particular species, the collections represent a more or less random sample of the slug species present at a site. Although the sampling technique and searching intensity were approximately the same at all sites, sampling of sites was not fully comparable because no attempt was made to sample each site at the same time of day or under the same weather conditions. Thus, differences in species activity patterns under different conditions may have contributed to differences in collection success for each species. However, with this caveat in mind, samples may give a crude estimate of relative species abundance at a site.

Pallifera varia was found only at the MLBS site and no other

species was collected there. Pallifera varia is known only from Virginia (Hubricht, 1953), but the absence of the other species from this site cannot be readily explained by differences in geographic range or elevation even though this was both the most northerly site and the highest (Table 1, page 7). Philomycus carolinianus is known to range from Canada to the Gulf Coast in eastern North America (Chichester and Getz, 1973) and has been collected from above 1200 m (Pilsbry, 1948). Philomycus venustus is known from Virginia to Georgia and from 1700 m in Virginia (Hubricht, 1953) to about 300 m (this study). Megapallifera mutablis has been found from Louisiana (Hubricht, 1956) to New York (Lee Fairbanks, pers. com.). Thus, the geographic and elevational ranges of all four species overlap and include the MLBS site and the reason for the apparent absence of the other species from MLBS is not evident. While Philomycus carolinianus was found at every site in Tennessee, Philomycus venustus occurred regularly in only two geographic groups (Clinch Mt. and Powell), rarely in one (Chilhowee Mt.) and not at all in the fourth (UT Woodlot).

There are at least three possible explanations for the differences observed. One is a difference in collecting success due to differences in time of day, weather, or other collecting conditions. Examination of collection data and field notes revealed no obvious factors but my field notes contain only qualitative observations of conditions. The precise measurements of environmental conditions that would be necessary for an analysis of

variance to resolve this question were beyond the scope of this study. The second explanation is that the differences are artifacts of chance and history. These animals occupy aggregated and ephemeral habitats and have low powers of dispersal. Chance local population crashes could temporarily change the relative abundance of a species at a collection site. While this may account for the observed differences in distribution, it is not possible to infer historical events from distributional data collected at a single point in time. The third explanation is that there is a real difference in distribution due to some difference in habitats. For example, Megapallifera mutablis was found at only one site (P01) in Tennessee. There it occurred with Philomycus carolinianus and Philomycus venustus. This site was unique among the collection locations in that it had been crudely maintained as a lawn. All the other sites were heavily wooded and this difference may account for the absence of Megapallifera mutablis at the other sites. The differences in abundance of Philomycus carolinianus and Philomycus venustus at other sites similarly may be accounted for by habitat differences among sites. No quantitative measurements of ecological conditions at the sites were taken and some ecologically important differences probably went unnoticed. There is very little literature on the ecology of these animals and information on their natural history is limited (Pilsbry, 1948; Ingram, 1949; Karlin, 1961). Although the cause of the differences in relative species abundance cannot be determined with certainty, differences in habitat preferences probably is the best explanation for this phenomenon.

Interspecific Genetic Similarity

The taxonomy of the Philomycidae, as with many molluscs, is relatively unsettled. Some authors (e.g. Pilsbry, 1948; Chichester and Getz, 1973) recognize as few as six species in this family while others (e.g. Hubricht, 1973; Branson, 1980) recognize as many as 14 species. The data collected for this study provide a good opportunity to compare the taxonomy based on morphological characteristics with the observed genetic relationships among the species. The taxonomy used in this paper is based in part on the work of Hubricht (1973), and the averaged genetic identity coefficients observed (Table 8, page 23) conform well to his morphological identifications. Interspecific comparisons between Philomycus carolinianus and Philomycus venustus showed an average genetic identity of 0.775 which is typical for congeneric species (Avise, 1974). The intergeneric comparisons among Philomycus, Pallifera, and Megapallifera ranged from 0.144 to 0.588, which also is typical for confamilial genera (Avise, 1974). Thus, to this extent, the genetic data are in good agreement with the conventional systematics.

Geographic Variability in Philomycus carolinianus

The genetic heterogeneity of allele frequencies among demes was significant at all the robustly polymorphic loci examined in Philomycus carolinianus (Table 9, page 26). The observed levels of heterogeneity are high; however, similarily high levels of heterogeneity have been found in other animal species as well

(e.g. Selander, 1970; Powers, 1975). The habitats at all of the collection sites were at least superficially very similar. Although it is possible that differential selective pressures occur at different sites, identification of possible selective factors at different sites was not attempted in this study. Recognizing this limitation, there is no apparent pattern to the heterogeneity that would suggest selection is causing the demes to differ. In these data, the same pattern is seen at every robustly polymorphic locus. Differential selection could produce differences in allele frequencies among demes at one or a few loci, but each locus would usually respond to an independent selective pressure. However, a group of functionally related enzymes might be responding to the same ecological condition. This does not seem likely in this case because the highly variable enzymes examined are of three different functional types (esterases, peptidases, and enzymes involved in glucose metabolism). The more parsimonious explanation is that heterogeneity is the result of some population phenomenon that promotes differentiation among demes.

Given the high genetic identities among demes of Philomycus carolinianus, it is difficult to distinguish fine scale relationships between demes. However, there appears to be a tendency for members of geographic groups to cluster together (cf. Figure 2, page 25). This suggests that the heterogeneity is related to geographic distribution. Table 10, page 27, shows that, on average, each deme was significantly more similar genetically to other demes

in the same geographic group than to demes in other geographic groups. This is what would be expected if the heterogeneity were due to restriction of gene flow over distance. Individuals may interbreed more or less freely in a small area, with a moderate amount of gene flow to nearby groups, and very little gene flow to distant groups. Adjacent demes also may have a greater likelihood of having been part of a single continguous population in the recent past with subsequent isolation of the groups.

Table 11, page 29, shows that the two groups of demes on one side of the river are more similar to each other than they are to the groups of demes on the other side of the river regardless of geographic distance. For example, the Powell demes are more similar on average to the Clinch Mountain demes (mean I = 0.971) than to the UT Woodlot demes (mean I = 0.925) even though Powell is over twice as far from Clinch Mountain as it is from the UT Woodlot (43 km vs. 16 km). This suggests that the Tennessee River is a major barrier to gene flow among these populations.

There are at least three possible explanations for the lack of a significant relationship in the linear regression of genetic distance against geographic distance. First, it may be a problem of scale and sample size. The within group distances range from approximately 0.3 to 2.4 km and the among group distances range from approximately 16 to 58 km, with only four observations of among group distances. The within group distances may be too small and the among group distances too large with too few

observations, to allow detection of any relationship. Second, a uniform decline in migration rate (exponential, linear, etc.) is an underlying assumption of this linear regression. Migration rate, however, may not decline uniformly with distance. This can be produced, for example, by social behaviors such as dispersal in which juveniles migrate a certain minimum distance from their natal area before breeding. Such a pattern of dispersal would result in near neighbors that are no more genetically similar than more distant neighbors. Third, allozyme data simply may not have the precision necessary to detect such a relationship. The resolving power of allozyme data is relatively low (Ayala, 1982) and while genetic similarity indices based on such data can easily distinguish taxonomic levels, the differences among conspecific demes may not be enough in many cases to produce unambiguous distinctions. It is difficult to distinguish among the three possible explanations without more data, and some combination of them actually may be operating.

Using Wright's hierarchical F-statistics (Wright, 1978), geographic subdivision of the population accounts for approximately 9% of the total diversity (Table 12, page 29). Of this 9%, 53.4% is due to differences among demes within geographic groups, 24.5% is due to differences among geographic groups within regions and 22.1% is due to differences between regions on opposite sides of the river. This shows that differentiation between demes is greatest at the smallest geographic scale. Since geographic distance appears

to be less important at the higher scale of among groups, one interpretation is that the distance between demes within a geographic group is sufficient to produce a high degree of differentiation among these demes. In addition, the barrier presented by the river accounts for a relatively high percentage of the total differentiation and it represents a barrier to gene flow that is unrelated to distance.

The high degree of interpopulational differentiation observed in Philomycus carolinianus demonstrates that these animals are not a panmictic population across their range but that their populations are highly fragmented. The evolutionary consequences of such a population structure include a higher incidence of inbreeding and a higher susceptibility to genetic drift through isolation even though these animals outcross. Although the variability within a deme is reduced by such a structure, the overall variability of the species remains quite high. Occasional fusions of previously isolated demes or bursts of migration would serve to maintain the variability on which selection acts.

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