SOCIAL SUBSTRUCTURE AND DISPERSION OF GENETIC VARIATION IN THE YELLOW-BELLIED MARMOT (MARMOTA FLAVIVENTRIS)

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Abstract--Yellow-bellied marmots located in the East River Valley and North Pole Basin of Gunnison County, Colorado were the subject of population studies for 16 years and 5 years, respectively. The detailed knowledge of individual life histories, demography, and colonial substructure of these animals provided an ideal background for a study of the significance and dynamics of electrophoretic variation in this species. Thirty specimens were collected from each of three localities at 2680, 3170, and 3660 m elevation, 203 blood samples were obtained from the East River Valley (2900 m), and 88 blood samples were obtained from North Pole Basin (3400 m). Allozyme variation was found at 8 of 20 loci examined. Significant positive results included an association between TRF genotype and aggressiveness, a correlation between LAP gene frequency and density, gametic disequilibrium between three pairs of loci, and significant heterogeneity in the dispersion of genetic variation among the colonies of marmots. No association between gene frequencies or heterozygosity and altitude, habitat, age, sex, survivorship, litter size, and a suite of behavioral variables was found. Recent mutation was excluded as an explanation of the distribution of genetic variation. Identical selective forces or sufficient gene flow among the five study sites could account for their similarities in gene frequencies and heterozygosity. Deterministic forces were not of sufficient magnitude to prevent significant drift acting within the spatial and temporal structure of marmot colonies.

INTRODUCTION

Population structure, substructure, and their interactions with other deterministic and stochastic forces are important considerations of population genet-

ic theory. Endler's (1977) models for clines in gene frequency had population structure as an essential element. Group selection theory, particularly that part dealing with kin selection, requires that drift acts within small, substructured populations (Wilson, 1975:107). Mutations in large, panmictic populations face a high probability of loss after only a few generations (Spiess, 1977:374). Hence, Wilson et al. (1975) deduced that the social substructure of mammalian populations (bottlenecking) allowed the rapid incorporation of chromosomal mutations into populations and the rapid evolution of mammalian taxa. Sampling errors in the association of gametes and inbreeding in small substructured populations greatly increased the probability of fixation of a mutation through drift, conditions that also lead to genetic heterogeneity between subgroups in the population.

Genetic heterogeneity among small, social groups, and by analogy the establishment of the effective action of kin selection in populations, is enhanced under the following conditions (McCracken and Bradbury, 1978): (1) preferential recruitment of juveniles into their natal colony; (2) the restriction of mate selection to those within the social group; and, (3) a low exchange rate between members of groups. McCracken and Bradbury (1978) found no heterogeneity in three allozyme loci in social colonies of a phyllostomid bat. All juveniles dispersed from the natal colony, and recruitment into harems was random. They concluded that sociality in this species was not due to kin selection.

The yellow-bellied marmot (Marmota flaviventris) is a large, herbivorous, diurnal, ground squirrel living in the mountainous regions of western North America. Sociality in a population of marmots located in the East River Valley (ERV) of Gunnison County, Colorado has been the subject of 16 yr of study by Armitage. Marmots were trapped, ear tagged, and marked with a black fur dye for individual recognition. Social relations within colonies were observed for 250 hr or more each summer. Marmots generally occupy a habitat of large rocks associated with nearby meadows; such habitat is patchy in the Transition Zone vegetation of the East River Valley. Smaller patches termed satellite sites are occupied by one or a few marmots; whereas in larger patches socially structured colonies of one or more polygynous groups occur. Satellite marmots are characterized by rapid

turnover in occupancy, poorer reproductive success, and lack of social structure when compared to colony animals (Svendsen, 1974). The typical polygynous group consists of a territorial male, a harem of two or three females, yearlings, and young of the year (Downhower and Armitage, 1971). Six colonies (in this paper colony is synonymous with polygynous group) in the present study (Nos. 1, 4, 6, 7, 8, and 10 in Fig. 1) were observed annually as typical marmot societies (Armitage and Downhower, 1974) though population density and social behavior varied from year to year (Armitage, 1975, 1977). Four additional areas, not under close observation, were designated as colonies for this study because they contained numbers of individuals and the stability of occupancy typical of colonies.

Sociality in marmots was also studied for the last five years (Johns and Armitage, 1979) at a subalpine site in North Pole Basin (NPB) of Gunnison County, Colorado. The methods of study were the same as for the ERV colonies. In NPB and similar alpine areas suitable habitat was more continuously distributed than in ERV, resulting in colonies being more continuously distributed with the boundaries of adjacent colonies defined by social mechanisms rather than the boundary of a suitable patch. Existing detailed knowledge of the life histories, population processes, and social substructure of marmots from the two study sites provided the ideal background to describe the relationship of genetic variation to these variables. To provide a broader ecological context in which to view allozymic variation in ERV and NPB populations, we collected additional marmots from elevations below the East River Valley, above North Pole Basin, and between the two areas.

MATERIALS AND METHODS

Blood samples were taken from 203 marmots in the East River Valley, elevation 2900 m and from 88 marmots in North Pole Basin, elevation 3400 m, of Gunnison County, Colorado during the summers of 1975 to 1977. A trapped animal was restrained in a handling sock and blood was collected from the femoral vein into a Vacutainer. Each animal was released at the site of capture. Blood samples were processed in the field and laboratory as described by Selander et al. (1971).

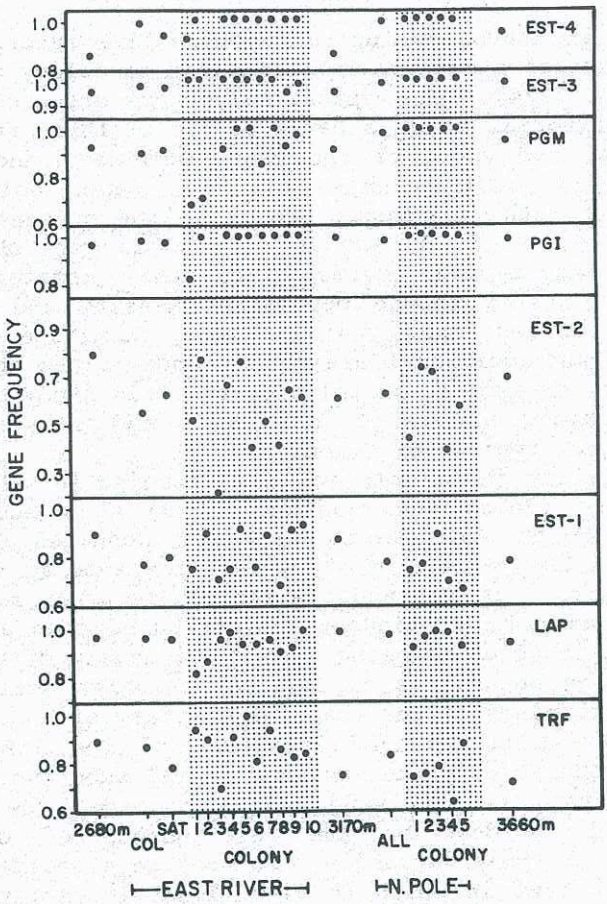

Fig. 1. Gene frequencies from eight blood allozyme systems from five study sites. The average gene frequencies from colonies (COL) and satellites (SAT) in the East River Valley study site are reported separately since they differ demographically. Gene frequencies from each colony in the East River Valley and North Pole Basin are in the shaded portions. Sample size for the three sites designated by altitude was 30. Sample sizes for TRF, LAP, EST-1 and 2 systems were: COL, 136; SAT, 67; ERV colonies 1 to 10: 8, 8, 7, 28, 8, 16, 8, 16, 11, 34; NPB all, 88; NPB colonies 1 to 5: 8, 15, 10, 10, 20. Sample sizes for the PGI, PGM, EST-3 and 4 systems, as for the above areas, were respectively: 118, 27, 6, 8, 0, 23, 8, 12, 8, 17, 8, 28, 58, 5, 15, 6, 5, 5.

Social Substructure and Genetic Variation

Ninety additional marmot specimens were collected in 1976 and 1977 by trapping or shooting from the following localities of Gunnison County, Colorado: 30 specimens from along the courses of Brush Creek and Cement Creek located 3.4 km and 7.5 km south of the town of Crested Butte at an altitude of 2680 m; 30 specimens from Schofield Park located 18.3 km north of Crested Butte at an altitude of 3170 m; and, 30 specimens from upper North Pole Basin located 18.4 km north, 9.8 km west of Crested Butte at an altitude of 3660 m. Samples of blood, heart, liver, and kidney tissues were taken from each specimen and frozen at -20 C. We attempted to randomize our sample of specimens by collecting at many different localities at the above sites. The five study sites represented an altitudinal gradient of approximately 1000 m and had marmots living in the Upper Sonoran, Transition, Canadian, Krumholtz, and Alpine life zones.

Horizontal starch-gel electrophoresis using a solution of 13% Electrostarch (Otto Hiller, Madison, Wisc.) was used for the analysis of all tissue proteins. The stains and buffer systems described by Selander et al. (1971) were used unless otherwise indicated; the phosphate buffer was described by Engel et al. (1970). Proteins were obtained from the following sources:

(1) Plasma--transferrin, two general protein systems, albumin, leucine aminopeptidase, and the two most anodal esterase systems. These esterase systems were scored by inhibiting the most anodal system with eserine sulfate (Selander et al., 1971).

(2) Hemolysate--6-phosphogluconate dehydrogenase (using buffer 4), hemoglobin (two systems, buffer 3), the two most anodal esterase systems (buffer 1) and the following systems using phosphate: phosphoglucose isomerase, phosphoglucomutase (two systems were scorable), lactate dehydrogenase (coded by two loci), α-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and NAD-dependent malate dehydrogenase.

(3) Other Tissue--glutamate oxalate transaminase (two systems for liver), sorbitol dehydrogenase (from liver using buffer 5 and stain 1 with sorbitol substituted for ethanol as the substrate), xanthine dehydrogenase (from liver), and the following systems using phos-

phate buffer: alcohol dehydrogenase (from liver), isocitrate dehydrogenase (two systems from heart and liver), α-glycerophosphate dehydrogenase (from heart), phosphoglucomutase (liver), and acid phosphatase (liver; Shaw and Prasad, 1970).

From 1963 to 1974 Armitage periodically collected and froze blood plasma samples for studies of serum hormones. These 185 samples had biochemically active leucine aminopeptidase, though the other plasma systems were not scorable. Our total sample size for leucine aminopeptidase was 567. Since we collected only plasma in 1975 and were unable to recapture some animals in 1976 or 1977, plasma system sample size was 203 for ERV marmots and 88 for NPB marmots, whereas hemolysate system sample sizes were 145 and 58, respectively.

RESULTS

The variable systems found in the blood were transferrin (TRF), leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and four esterases (EST-1 to 4), with two alleles at each locus. The gene frequencies and observed Hardy-Weinberg expected genotype frequencies are in Table 1. The variable tissue systems were α-glycerophosphate dehydrogenase (α-GPD) and phosphoglucomutase (PGM) with the variant allele found in only 1 of the 90 specimens for each protein (not the same animal). Tissue α-GPD and PGM were systems that were either not present or not scorable in hemolysate.

The two plasma esterase systems were in close proximity on the gel with the slow band (S) of EST-1, the most anodal of the two systems close to the fast band (F) of EST-2 so there was some difficulty in scoring these systems. Among 76 young in 31 litters in which both parents were known there were five phenotypes in the EST-1 system and two in the EST-2 system that were not consistent with a Mendelian interpretation of inheritance and these were regarded as scoring errors in a Mendelian system. The other six variable systems supported a Mendelian interpretation of inheritance without exception.

The proportion of polymorphic loci, loci with the frequency of the alternative allele greater than 0.01,

Table 1. Gene frequencies (p_1 and p_2) and observed (O) and Hardy-Weinberg expected (E) numbers of genotypes for blood allozymes for all marmots sampled.

System*	p_1	p_2	p_1p_1	p_1p_2	p_2p_2
TRF					
O	.831	.169	254	127	1
E			263.8	107.3	10.9
LAP					
O	.952	.048	515	49	3
E			513.3	52.3	1.3
PGI					
O	.989	.011	288	4	1
E			287.0	5.9	0.0
PGM					
O	.928	.072	257	30	6
E			252.5	39.0	1.5
EST-1					
O	.797	.203	230	149	3
E			242.7	123.6	15.7
EST-2					
O	.637	.363	138	211	33
E			155.2	176.6	50.2
EST-3					
O	.981	.019	283	9	1
E			282.1	10.8	0.1
EST-4					
O	.956	.044	267	26	0
E			267.6	24.9	0.6

*Abbreviations in text.

estimated from the blood systems was 0.40 (8/20) and from the blood and tissue loci together was 0.27 (8/30). Average heterozygosity ($H = \Sigma (1 - \Sigma p_i^2)/20$) was 0.0749, where p is the frequency of the ith allele at each locus. The average proportion of heterozygous loci per individual was 0.0872 (SE = 0.0034) calculated over 20 loci. To facilitate comparisons between marmots from the five study sites the rare tissue variants were not used in any calculations. Later in this paper the proportion of heterozygous loci was calculated as a fraction of the eight variable systems, thus removing a constant factor of 12 from these calculations, and these proportions were arcsine transformed to normalize their distributions (Sokal and Rohlf, 1969:386).

There were no significant differences in gene frequencies and heterozygosities for marmots at the five study sites shown in Figs. 1 and 2. The gene frequencies of ERV colonial and satellite marmots were reported separately to demonstrate their differences in demographics and sociability. To test for differing gene frequencies between study sites 95% confidence intervals were drawn around each gene frequency. Frequencies that did not overlap other confidence intervals were compared with a t-test of proportion. Gene frequencies for marmots from five study sites, compared two at a time for eight loci, dictated a probability level of $P < 0.012$ for the 80 comparisons to avoid a Type I statistical error (Sokal and Rohlf, 1969:156); no significant case at this level was found. A one-way analysis of variance (ANOVA) of individual heterozygosities showed nonsignificant differences among the five populations ($F_{4,377} = 1.31$; $P = 0.24$). Three loci, TRF, EST-1, and EST-2, showed large excesses of heterozygotes, possibly due to gametic disequilibrium among these systems (discussed later); the small excess of homozygotes shown in four systems indicated a low magnitude of Wahlund effect from pooling the gene frequencies for marmots from the five study sites (Table 1).

There were no significant differences in gene frequencies or heterozygosity between colonial and satellite animals in the East River Valley. However, genetic heterogeneity existed among colonies in both the East River Valley and North Pole Basin, as reflected by divergent gene frequencies (Fig. 1) and heterozygosities (Fig. 2).

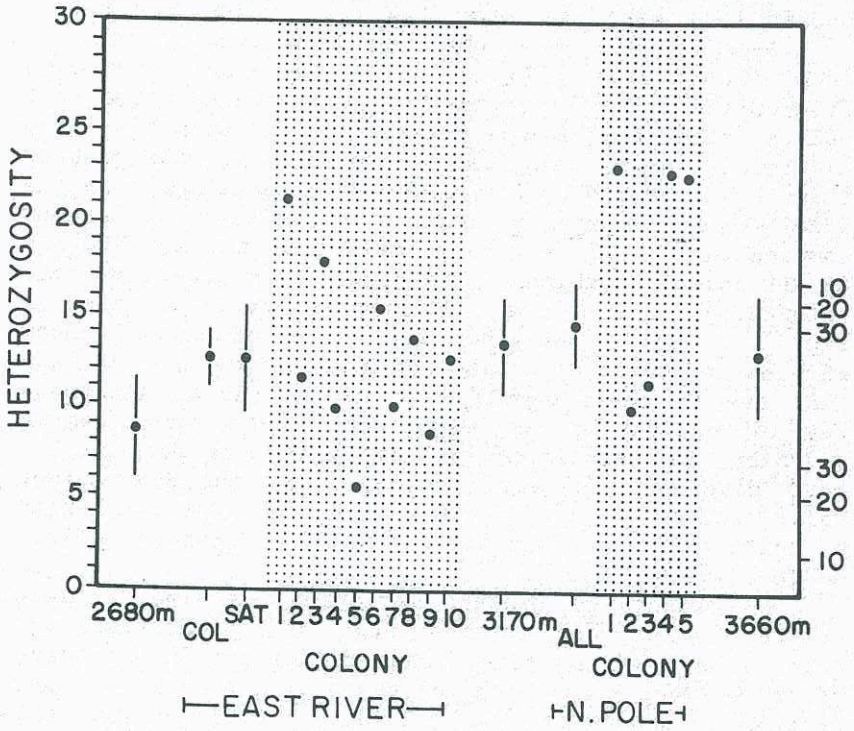

Fig. 2. Average individual heterozygosity from eight blood allozyme systems from five study sites. The legend for the X axis is as described in Fig. 1, and sample sizes were averages of those in Fig. 1. The highest and lowest heterozygosities from 1000 random samples of 10, 20, and 30 marmots are shown on the right. Vertical lines represent two standard errors around the mean heterozygosities.

The simplest prediction that could be made from a genetic comparison of colonies and satellites would be that colonies would be more homozygous with a greater variance than the approximately panmictic satellites because of drift from small effective population size, nonrandom mating, and the possibility of inbreeding. Such was not the case. The average heterozygosity of the two groups was not significantly different ($F_{1,201}$ =

0.05; $P > 0.75$), and a test of the equality of variances showed they were the same ($F_{62,141} = 1.04$; $P > 0.40$).

Genetic data were obtained from every member of each colony from 1975 to 1977; therefore, our gene frequencies and heterozygosities represented parametric values rather than sample estimates. To estimate if the divergence among colonies was of a magnitude greater than that produced by the random effects of small sample size alone, we used a computer to select 1000 random samples of 10, 20, and 30 animals from the 203 ERV and 88 NPB animals combined. The highest and lowest average heterozygosities of the 1000 samples are illustrated in Fig. 2. Points outlying the bounds of these random samples indicated divergence of a magnitude greater than that due to small sample size alone. As an alternate way of estimating the magnitude of divergence among colonies, we calculated similarity indices for the two most divergent colonies, colony 5 of the East River Valley and colony 1 of North Pole Basin. The similarity by Nei's (1972) measure was $I = 0.990$ and Rogers' (1972) measure was $S = 0.963$.

No measure of genetic variation was significantly related to sex, age, survivorship, or reproductive success. There was no significant difference in heterozygosity between sexes with the sexes from each study site treated as groups ($F_{9,372} = 0.90$; $P = 0.93$) or with the sexes from all sites pooled ($F_{1,380} < 0.01$; $P = 0.93$). There were no significant differences in gene frequencies between the sexes at the five study sites.

An ANOVA of the heterozygosities of marmots grouped as 0, 1, 2, and 3 or greater years of age showed no significant differences among these age classes in the ERV and NPB populations ($F_{7,283} = 1.8$; $P = 0.09$). In addition, an ANOVA of the data for the age classes pooled over the two populations showed nonsignificant differences ($F_{3,287} = 0.14$; $P = 0.93$), as did a test for overlapping confidence intervals of gene frequencies among age classes.

Survivorship from 0 to 1 yr of age was tested for association with genetic variation in ERV marmots born in 1975 and 1976 treated as a cohort. Data from the NPB populations were insufficient to replicate this analysis. Thirty-seven of 53 ERV young survived to one yr of age and were the basis for this analysis. The mortality rate from 0 to 1 yr of age, q_x from life table statistics, calculated for 507 ERV young born since 1962 was

0.609; hence survivorship through this age class is a critical period in a marmot's life history. A Chi-square test of the contingency of survivorship on genotype, number of heterozygous loci, and animals with low heterozygosity or high heterozygosity did not show significant association between the variables. For the analysis of high and low heterozygosity the sample was divided into two nearly equal groups, those with low heterozygosity (0 or 1 locus) and those with high heterozygosity (2, 3, or 4 loci).

The number of heterozygous loci (1 to 4 loci) of each of the 15 ERV female marmots that produced 28 litters was used to group litter sizes for an ANOVA. There was no significant difference in litter sizes among the groups ($F_{3,24} = 2.61$; $P > 0.07$). Data were insufficient to test for significant differences between genotypes for the female marmots or for the effects of parity, and too few territorial males fathered all the litters to allow a similar analysis for males.

There was little relationship between genetic variation of ERV marmots and their behavioral characteristics derived from mirror image stimulation (Svendsen and Armitage, 1973). The first five axes of a factor analysis (BMDP4M program; Dixon, 1975) of the frequencies of 22 behaviors recorded while each marmot was exposed to mirror image stimulation accounted for 56.7 % of the observed variation. These axes were identified as I. Sociable; II. Aggressive; III. Avoidance; with factors IV and V not named. One-way ANOVA's were calculated using genotypes, the number of heterozygous loci, and animals with high and low heterozygosities (as described for survivorship) as groups. The factor scores on the five axes were used as dependent variables. The only significant relationship showed transferrin-FF and -FS genotypes grouped on the second axis ($F_{1,172} = 6.25$; $P = 0.02$).

Animals heterozygous (FS) at the transferrin locus scored more negatively on the second axis than those with the FF genotypes; this relationship suggests that marmots with a heterozygous locus are more aggressive. We had no behavioral data on animals with SS genotypes. Similarly, absence of suitable sample size within groups precluded analysis of behavior and genotypes for PGI and EST-3 and 4. These 42 ANOVA dictated a probability level of $P < 0.025$ for significance to avoid a Type-I error.

There was a positive correlation between the minimum number of marmots alive and the frequency of the most common allele of LAP ($r = 0.48$; $0.05 < P < 0.06$; Fig. 3) in the East River Valley. Although the correlation was of marginal statistical significance, we decided to report this unique relationship since this same correlation was reported in cyclic microtine rodents (Gaines et al., 1978; Tamarin and Krebs, 1969). This density cycle can be partially dampened by the explanation of artificial mortality that occurred in this marmot population. Part of the first increase in density was due to new colonies being discovered and trapped. Approximately 10 animals were lost during the decline in density when marmots proximal to occupied cabins were removed at the request of the cabin owners.

The change in density was reflected in both the numbers of colonial and satellite animals trapped. To test the hypothesis that the cycle was a function of trapping success, we calculated a Jolly (1965) stochastic estimate. The upper bound of that estimate is at the top of the vertical lines in Fig. 3. The change in density was reflected in the Jolly estimate, colony and satellite portions of the population, and average colony size. Hence, the density change has a real element as well as the artificial ones discussed above.

Even though there was an artificial element of density change, its variation with the frequency of the Lap-F allele was not so readily explained. The frequency of that allele was relatively lower in three of the seven colonies trapped during the decline phase. Kohn and Tamarin (1978) and Tamarin and Krebs (1969) analyzed such a correlation with a plot of change in gene frequency Δp vs gene frequency p. They suggested, citing Li (1955), that a significant negative slope of Δp on p was a statistical test for a stable polymorphism with the population being perturbed and returning to its equilibrium gene frequency. Our evaluation of this technique led us to think it was an unsuitable test for a stable polymorphism. Nevertheless, our regression of Δp on p produced a significant positive slope ($m = 0.33$; $t = 2.42$; $P < 0.04$). Li's (1955) theory was developed for nonoverlapping generations, hence our regression violated that condition.

Significant gametic disequilibrium, nonrandom association of alleles in gametes, occurred between the TRF/EST-1 loci, TRF/EST-2 loci, and EST-1/EST-2 loci

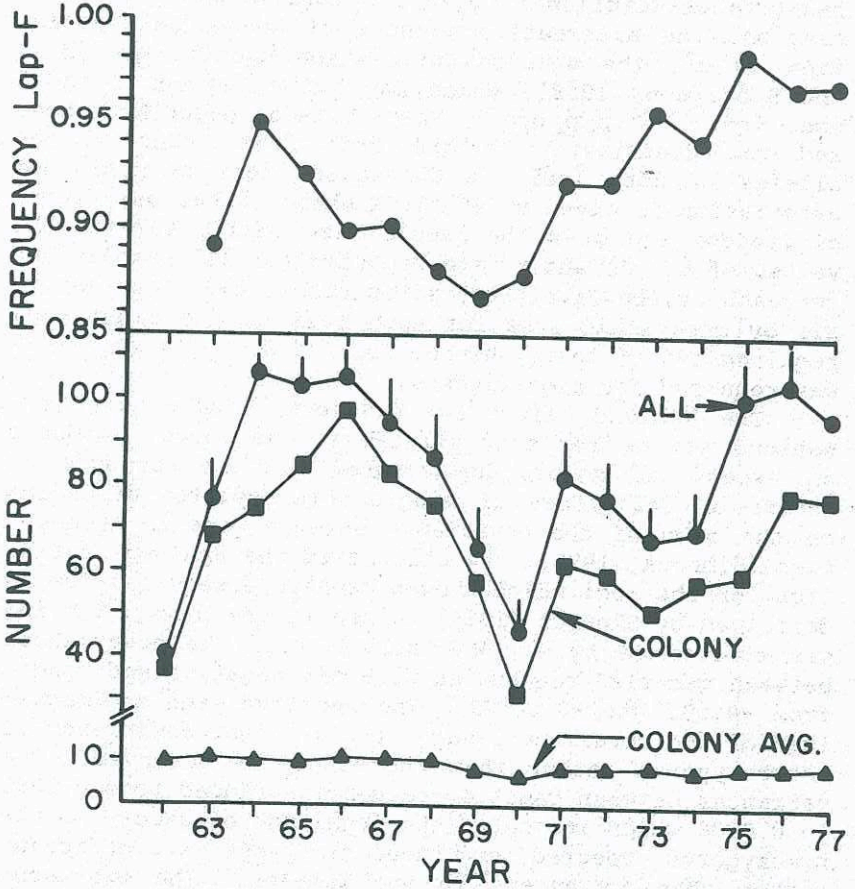

Fig. 3. Change of marmot density and the frequency of the Lap-F allele. The curves labeled colony and all (colony and satellite) represent minimum numbers alive, and the curve labeled colony average is the minimum number alive per colony. The vertical lines represent the upper bound of a Jolly (1965) stochastic estimate of numbers.

(Table 2). The linkage disequilibrium statistic, D, (Hill, 1974) measured the deviation from random association between alleles and ranges from -0.25 for maximum negative association to 0.25 for maximum positive association. An alternative measure of nonrandom association is r^2, the squared correlation coefficient (Hill and Robertson, 1968), which may range between 0 and 1 where $r^2 = D^2/(p_1 p_2 q_1 q_2)$, where D is as described above and the denominator is the gene frequencies of the alleles at both loci. A Chi-square test of nonrandom association is given by $\chi^2 = r^2N$ where χ^2 has one degree of freedom and N is the sample size (Hill, 1974). The values of D, r^2, and χ^2 are reported for all samples and for each of the five study sites (Table 2). This analysis by geographic area for each pair of the eight loci required 196 χ^2 tests; hence, for $P < 0.005$, a $\chi^2 > 7.87$ was required for significance.

The Wahlund effect for two loci, analagous to the Wahlund effect for four alleles at one locus, produces an excess of double homozygotes and can produce an excess or deficiency of single heterozygotes depending on the sign of the covariance between gametic frequencies (Sinnock, 1975). We calculated the Wahlund statistics for the loci which showed gametic disequilibrium as described by Sinnock (1975) to see if the disequilibrium was confounded by the Wahlund effect. The covariances between gametic frequencies were all negative and ranged from -0.0027 to -0.00023. The negative sign and magnitude of the covariances suggested a slight deficiency of heterozygotes rather than the excess we observed. The variances between gametic frequencies ranged from 0.0053 to 0.0086 which indicated the magnitude of excess double homozygotes expected, and these genotypes were deficient rather than in excess in our samples. The two-locus Wahlund effect was of small magnitude and produced effects opposite of those necessary to account for our observations.

We have no way of causally explaining the observed gametic disequilibrium, but the genetic divergence due to drift within the colonies suggested that drift may also have given rise to the observed disequilibrium. We divided the ERV population into two sections, with those animals living in the southeast end of the valley as one group and those living in the northwest sections as the other group, and then calculated the measures of gametic disequilibrium for those samples (Table 3). The distri-

Table 2. Measure of linkage disequilibria (D), squared multiple correlation coefficients (r^2), and χ^2 tests for significant gametic disequilibria were calculated for marmots from the five study sites and for all sites pooled.

Systems*	Measure	2680 m	E. River	3170 m	N. Pole	3660 m	Total
TRF/EST-1	D	-0.0040	0.0227	0.0080	0.0390	0.0051	0.0222
	r^2	0.0024	0.0236	0.0036	0.0700	0.0007	0.0222
	χ^2	0.07	4.47	0.61	6.22	0.02	8.47***
TRF/EST-2	D	-0.0152	0.0388	0.0397	0.0395	-0.0104	0.0181
	r^2	0.0196	0.0488	0.0396	0.0699	0.0026	0.0104
	χ^2	0.06	9.91***	1.20	6.22	0.08	3.95
EST-1/EST-2	D	0.0047	0.0648	0.0632	0.0401	-0.0104	0.0800
	r^2	0.0020	0.1006	0.1554	0.0515	0.0031	0.0634
	χ^2	0.06	20.42***	4.66	3.70	0.09	24.21***
Sample Size		30	203	30	88	30	382

*Abbreviations in text; *** $P < 0.005$.

Table 3. Measures of gametic disequilibrium calculated after subdividing the marmot samples from the East River Valley into southeast and northwest sections. Systems notations are the same as in Table 2.

Systems	Measure	East River Valley	
		Southeast	Northwest
TRF/EST-1	D	0.0204	0.0175
	r^2	0.0176	0.0185
	χ^2	1.92	1.11
TRF/EST-2	D	0.0623	0.0254
	r^2	0.1141	0.0292
	χ^2	12.44***	1.74
EST-1/EST-2	D	0.0519	0.0989
	r^2	0.0694	0.2166
	χ^2	7.56**	12.99***
Sample Size		109	60

**$P < 0.01$; ***$P < 0.005$.

bution of the rare alleles of PGI and PGM which were largely found in southeast colonies suggested this division. Large sample size is typically required to detect gametic disequilibrium (Brown, 1975a), thus preventing a finer scale of analysis. The results of this division suggested that local population parameters were an element of the observed gametic disequilibrium. Between TRF/EST-1, disequilibrium was significant only in the southeast colonies. Also, the magnitude of the EST-1/EST-2 disequilibrium was different in the two groups.

DISCUSSION

The significant positive results of this study included an association between transferrin genotype and aggressiveness, a weak correlation between Lap-F gene frequency and density, gametic disequilibrium between three pairs of loci, and significant heterogeneity in dispersion of genetic variation through the colonies of marmots. There was no association between genetic variation and altitude, habitat, age, sex, survivorship, litter size, and a suite of other behavioral variables. The presence of each allele of the eight loci at each study site (except for the rare PGI missing from the 3660 m sample) indicated that recent mutation was not an explanation for the distribution of genetic variation. The similarity of gene frequencies among study sites could be accounted for by identical selective forces in each area or a sufficient rate of gene flow among areas.

Our analysis was statistically conservative compared with other papers attempting similar correlations between genetic variation and life history and environment. Such conservatism was justified considering that in excess of 1000 statistics were calculated in the analyses presented. A model by Dickinson and Antonovics (1973) showed that such correlations may provide evidence of the action of selection on finite populations, but stochastic elements can significantly vary the observed correlation through time. Clarke (1974) considered ecological methods valuable in the detection of selection. The general lack of such correlations in our study may be accounted for by theories that consider the relationship of genetic variation and environmental "grain" (Soule, 1976; Selander and Kaufman, 1973a). The theory suggested that organisms rely less on genetic mechanisms to cope with environmental change as they become larger and more capable of homeostatic control, i.e., the organism experiences the environment as fine-grained. The marmot is a relatively large mammal, hibernates for seven months of the year, and is generally restricted to a habitat of rocky meadows; hence, it probably experiences the environment as fine-grained. The theory of grain also predicted that fine-grained organisms should tend toward a single phenotype (Selander and Kaufman, 1973a), but the level of variation in marmots was typical of that of rodents (Selander, 1976). Selander and Kaufman (1973a) suggested that

there was a threshold size beyond which heterozygosity does not decrease; perhaps the marmot is beyond that limit.

Gaines et al. (1978) reviewed the literature relative to the commonly reported covariation of gene frequency and population density in mammals. Such correlation, apart from a direct causal relationship, could be due to the linkage of an allozyme locus to loci subject to selection. Models by Charlesworth and Giesel (1972) accounted for such covariation as side effects of demographic change.

Linkage between a neutral and selected allele would be subject to rapid decay, and nonrandom association due to drift would vary in its direction and magnitude through time (Hedrick et al., 1978). Hence, linkage and drift do not satisfactorily account for this phenomenon for many species in widely scattered geographic areas. The simplest hypothesis that would explain our observation would be chance variation of gene frequency in the same direction in several marmot colonies through the population processes relative to each colony. A more complex hypothesis would be that of density-dependent or frequency-dependent selection acting on semi-isolated colonies over the 5 km that separate the most distant colonies.

The positive relationship between aggressiveness and transferrin genotype could be causal or linked, as discussed for density, or a spurious statistic. We have no way of choosing among these hypotheses. Our study of the relationship of genetic variation and behavior was generally comparable to that of Garten (1976) on the old-field mouse, Peromyscus polionotus. Numerous behavioral acts were correlated with heterozygosity in geographic samples. Garten's suggestions, that attack time, food control behavior, and social dominance are more than 96% predictable from the variation found in soluble tissue proteins, were unsatisfying to us. We suggest that these correlations were artifacts of his statistical analysis. Specifically, Garten regressed average heterozygosity as the independent variable on the average behavioral score for each of five study sites. Linear and polynomial regressions, fitted to five pairs of variables which were averages, could account for the reported predictability. A regression model with more than one value of Y per X would be more appropriate (Sokal and Rohlf, 1969:430). In this model

an ANOVA is calculated for the groups of Y variables; given a significant ANOVA, a regression of X on the Y variables (rather than the average of Y's for groups) may be calculated.

An explanation of gametic disequilibrium found in nature is frequently difficult (Clarke, 1974). Disequilibrium may be caused by migration, selection, mutation, and drift in substructured populations (Nei and Li, 1973). Population substructure was implicated as an element of the gametic disequilibrium in ERV marmots. Gametic disequilibrium was reported between esterase loci in barley (Kahler and Allard, 1970), fruit flies (Baker, 1975), and salamanders (Webster, 1973). Chromosomal mapping of the involved esterase loci in barley and fruit flies showed they were closely linked and that the association probably arose by gene duplication. Webster also hypothesized gene duplication due to the similar relative mobilities of the allozymes on starch gel. The marmot EST-1 and EST-2 alleles were also in close proximity on starch gels. Hence, gene duplication and population substructure may be elements of the gametic disequilibrium we observed. The observed excess of TRF, EST-1, and EST-2 heterozygotes may be related to this nonrandom pattern of association.

Though the above correlations and gametic disequilibrium may represent the action of deterministic forces on genetic variation, the magnitude of these forces was insufficient to prevent significant drift within colonies. This heterogeneity between colonies was the significant finding of our study. The relationship of drift to demographic processes among colonies is detailed elsewhere (Schwartz and Armitage, 1980). Demographic processes may enhance group heterogeneity; greater than 60% of harem residents were recruited from their natal colony. The Mendelian pattern of inheritance of allozymes suggested no mate selection outside the social group. Only 40 of 790 marmots observed since 1962 moved between colonies, and only 15 of these movements probably resulted in gene flow. Drift among colonies was of greater magnitude than that expected due to random sampling alone. A Nei's (1972) index of 0.989, calculated for the most divergent marmot colonies, is comparable to the level of differentiation seen at the subspecies level in fruit flies (Avise, 1976). A Rogers' (1972) index of 0.963 is comparable to that seen between closely related species of Spalax and Thomomys

(Nevo et al., 1974) and Spermophilus (Cothran et al., 1977), although the average of mammalian sibling species is 0.81 (Avise, 1974).

The functional significance of allozyme variation in mammalian populations is little known, hence the ecological and evolutionary meaning of the genetic divergence shown among marmot colonies is difficult to assess. Though we cannot exclude the action of selection on the observed allozyme variation, its magnitude is less than that of drift. Thus, the genetic variation found in our study functions best as marker alleles to be studied relative to population processes.

The significance of regulatory gene and chromosomal evolution in mammalian populations has received increasing attention (Cherry et al., 1978; Wilson et al., 1975; King and Wilson, 1975), and the impact through evolutionary time of heterogeneity due to the substructure of a population is perhaps best considered in the context of regulatory genes and chromosomal changes. The dynamics of genes through long periods of time require the consideration of the actions and interactions of the many deterministic and stochastic forces that affect gene frequency. A contrast in the alternate results of the forces affecting genetic heterogeneity might be found in considering the 11 subspecies of yellow-bellied marmot and the over 400 subspecies of the gopher Thomomys found in western North America (Hall and Kelson, 1959). We offer the following speculation on the dynamics of marmot genetics.

The models of Levin et al. (1969) relate to the conditions found among colonies of marmots. Their models for maintaining t alleles in small demes of house mice showed rapid drift to fixation for the T allele in unconnected demes. Small demes, approximating the effective population size of marmot colonies, interconnected by gene flow of 3% were effective in preventing the loss of the t allele for 200 generations. A model of drift and gene flow (Endler, 1977) between demes (of 100 individuals exchanging genes at the rate of 0.20) may represent elements relative to marmot populations if the East River Valley is considered as a genetic unit. Endler's model yielded a wide range of gene frequencies through 1000 generations with pronounced area effects and great heterogeneity between areas. The ERV contains patches of suitable habitat with structural and biological features such as cliffs, large expanses of

forest, and a broad river floodplain which may serve as partial barriers to gene flow between suitable habitat patches and between the more continuous alpine habitat. Given that the substructure of a marmot population like that of the ERV is an element retarding the loss of genetic variation and that heterogeneous populations in semi-isolated areas exchange genes, the potential for retaining genetic variation in the population by drift for long periods of time is great.

The well-known social structure of larger mammals, e.g., langurs (Blaffer Hrdy, 1977) or lions (Bertram, 1977), and the increasing evidence for substructure in small mammal populations suggest the generality of the genetic findings of our study. Those considering the rate of mammalian evolution, evolutionary ecology of mammals, and social evolution may continue to look at population substructure as a valuable element of their arguments.

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