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GENETIC DIFFERENTIATION BETWEEN POPULATIONS OF THE ANT APHAENOGASTER 'RUDIS' IN THE SOUTHEASTERN UNITED STATES

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Chromosomal and isozyme variation was examined in populations of the ant *Aphaenogaster 'rudis'*. Georgian populations of this ant are referable to a lighter Coastal Plains or a darker Montane phenotype. Coastal Plains populations are fixed or nearly fixed for a null esterase allele, Es^0, whereas high activity allele frequencies characterise Montane populations. High *Mdh-a*^{1} frequencies (> 0.5) are typical of Coastal Plains populations, but in Montane populations this allele is rarer (< 0.3) or absent. Study of a narrow (approximately one kilometer wide) contact zone (Turner's Corner) between these two forms showed that Coastal Plains ants have n = 20 as against n = 22 for Montane ants there, that there was no chromosomal evidence of hybridization, but that the presence of low frequencies of the alternate *Es* alleles in both forms indicates that there is some introgression. The Montane phenotype is itself heterogeneous at Black Rock Mountain, where a further, 18-chromosome, cytotype shows marked microhabitat segregation from the sympatric 22-chromosome form and differs from it in allele frequencies at all four loci examined (*Mdh-a, Mdh-b, Es, Amy*). Average relative genetic distances (Nei's D_m) between populations within all forms are low (maximum of 0.03 ± 0.01) compared with interform distances (minimum of 0.19 ± 0.03), which are similar to distances between the *'rudis'* forms and both of *fulva* and *treatae*. *Aphaenogaster fulva* has a very similar karyotype to that of 18-chromosome *'rudis'*, in which some specimens show weak development of the taxonomic characters distinguishing *fulva* from *'rudis'*. However, *fulva* and 18-chromosome *rudis* differ markedly allozymically, especially in that *Amy*^{5} and *Amy*^{6} are the only amylase alleles in *fulva* and *Amy*^{4} is the only amylase allele in 18-chromosome *'rudis'*. An Alachua County, Florida *rudis*-group population has a 22-chromosome karyotype different to that of Georgian *'rudis'*, but the genetic distance values suggest it may be most similar genetically to the Montane 22-chromosome form. Low-level gene flow between *fulva* and *rudis*-group populations is suggested by similarity of *fulva* and two different sympatric *rudis*-group populations in terms of *Es* alleles present.

The Georgian 20- and 22-chromosome *'rudis'* karyotypes may possibly be related by Robertsonian changes, but the relationship between these and the 18-chromosome forms (18-chromosome *'rudis'*, *fulva*, and an 18-chromosome Florida isolate), *treatae* (n = 21), and *lamellidens* (n = 19), are obscure. A non-Robertsonian chromosome number polymorphism was found in one Coastal Plains population.

No unequivocal evidence was found for an overall departure of genotype frequencies from those expected under the Hardy-Weinberg Law.

The *'rudis'* cytotypes in Georgia are sibling species whose close morphological resemblance may reflect ecological but not genetic similarity.

Introduction

Aphaenogaster rudis is a commonly-found ant in hardwood and mixed pine-hardwood woodlands in the eastern United States. The colonies nest inconspicuously under stones, in rotten wood, in leaf litter, or deep in the soil, depending upon the season. The available evidence is consistent with each colony con-

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sisting of a single, once-mated queen and her worker progeny (Crozier, 1973), with male-production being through queen-laid eggs except where the queen has probably died (Crozier, 1974).

Because hymenopteran males are haploid, the genotypes of the parental queen and male responsible for each colony can usually be determined through examination of their worker progeny (for a review of hymenopteran genetics see Crozier, 1975). Male haploidy in this situation allows the determination of gametic combinations, something that is not so easily done for species with both sexes diploid (except, of course, for sex-linked loci). Apparent strong gametic phase imbalance was indeed found in one '*rudis*' population but, as will be seen below, a simple alternative explanation to intraspecific selection was found.

The genus *Aphaenogaster* is well-developed in North America, with Creighton (1950) listing 18 species. *A. rudis* is the commonest species at most localities in the eastern U.S., and shows considerable colour variation from one region to another, as will be discussed below. One key question addressed by this paper is whether *rudis* is in fact a single specific entity, or several.

Methods

Electrophoretic techniques

Individual ants were ground in a drop of gel buffer together with a small amount of ground glass in depressions in a Delrin block using a Delrin grinding rod (Johnson, 1966; Bush & Huettel, 1972). The homogenate from each ant was absorbed into one or two rectangles of filter paper (Whatman number 3MM) and inserted into one or two gels, as required. Most individuals were thus scored for all four loci.

The malate dehydrogenase and esterase systems were examined using starch gels and the same buffers (tris-citrate, pH. 7.0) as described previously (Crozier, 1973). Amylase separations employed 5% acrylamide gels prepared using Cyanogum-41 (Fisher Scientific), in a tris-citrate buffer. The pH of the cyanogum stock solution was adjusted to 7.5; polymerization was by the TMED-ammonium persulphate system. The same electrode buffer was used for amylase separations as for starch gels. Whereas starch gels were electrophoresed for 5-6 hours at 190 volts, acrylamide gels were run for 12-13 hours at 110 volts potential difference.

Visualization of malate dehydrogenase was as previously described. For esterase, the substrate solution was 50% acetone-water in which was dissolved α- and β-naphthyl acetates (each 1g/100 ml). Three ml of substrate solution were added to 100 ml of 0.1 M K-phosphate buffer (pH 6.7), and 0.1 g of Fast Blue RR was stirred rapidly into this solution just before it was poured over the sliced gel. After one hour's staining at 37°C in the dark for esterase and 2-3 hours for malate dehydrogenase, gels were washed and fixed. Sliced amylase gels were soaked for six to eight hours at room temperature in 0.02 M K-phosphate buffer, pH 7.3, containing soluble starch (10 g/l). The gels were then washed in two changes of tap water, immersed in potassium tri-iodide (Lugol's solution) for 10-15 minutes, washed, and fixed. The amylase bands appear as clear zones on a blue background (Scandalios, 1969).

The electrophoresis was performed in low-temperature incubators (Freas #815). Amylase mobilities proved to be thermolabile, requiring temperature maintenance to be as low as practicable (2°C).

Twenty-four ants were electrophoresed per gel, of which four were standards of known genotype chosen chiefly to aid in scoring amylase gels. At least eight ants were electrophoresed per colony, with the probability of misclassifying heterozygote queens as homozygotes therefore being $p = .0078$, on the assumption of Mendelian ratios.

Chromosomal techniques

Pupae and prepupae were pierced with sterilized minuten and placed on colchicine-Ringer (0.005%) droplets so that the colchicine could diffuse into them, and left for 10-15 hours. Brains or testes, or both, were dissected out under 1% sodium citrate, and the organs transferred to fresh citrate for 10-20 minutes. The organs were then fixed in acetic-methanol (1:3) for 30-60 minutes, before being transferred to 3-4 drops of 60% acetic acid on a clean warmed slide. If necessary, the organs were then macerated using mounted minuten, but usually dissociation was rapid and spontaneous. The suspension was allowed to dry on the slide, which was kept on a warm slide-warmer until this had occurred; the slide was tilted occasionally so as to prevent the suspension drying all in the one place.

The preparations were stained either using lacto-acetic orcein (Crozier, 1970) or Giemsa (Gurr's R66 diluted 1:24 in pH 6.8 buffer made from a Gurr's buffer tablet). Orcein-stained slides were mounted in Euparal, Giemsa preparations in Permount.

Deposition of voucher specimens

Three mounted worker specimens for each colony examined have been deposited in the Museum of Comparative Zoology of Harvard University, Cambridge, Massachusetts.

Results and analysis

Inheritance patterns

Mdh-a and *Mdh-b* both have two codominant alleles with heterozygotes possessing a third band of intermediate mobility (Fig. 1), indicating a polymeric, probably dimeric, structure for these enzymes.

Es phenotypes include those referable to genotypes involving three active alleles and also a null phenotype (Fig. 1). *Es*-negative individuals do not lack esterase activity; *Es* bands appear within an hour but gels left to stain longer develop many more bands in complex overlapping patterns. The model of inheritance adopted for this locus is that *Es*-negative individuals are homozygotes for a recessive null allele; this is the simplest model to fit the data. Other more complex models also fit the pattern of inheritance observed, such as two models (differing by whether the null pattern is dominant or recessive) involving a regulator locus. Colonies containing null individuals as well as others showing two of the active alleles could enable distinction between the three models mentioned; collections were made at localities where such colonies might be found, such as Black Rock Mountain, but the search was unsuccessful. Active *Es* alleles are codominant, with no hybrid band in heterozygotes. Faster accessory bands occurred in some individuals, probably indicating either differences in aggregation rates of the molecules, or conformational differences.

Amy phenotypes (Fig. 1) indicate the occurrence of at least six codominant alleles, lacking hybrid bands in heterozygotes. Slight differences in mobility within apparent allele classes indicated that these alleles are certainly 'electromorphs' (King & Ohta, 1975) rather than true unitary alleles. Because of difficulty with obtaining a sufficient supply of standard ants for the rarer alleles Amy^1 and Amy^6, at some localities scoring of these and other alleles of similar mobility was only possible as Amy^1 or Amy^2, Amy^5 or Amy^6. Therefore, the frequencies of the two pairs of alleles of extreme mobility are lumped in Table 2 and for the preparation of Tables 3 and 4, and Figs. 6 and 10.

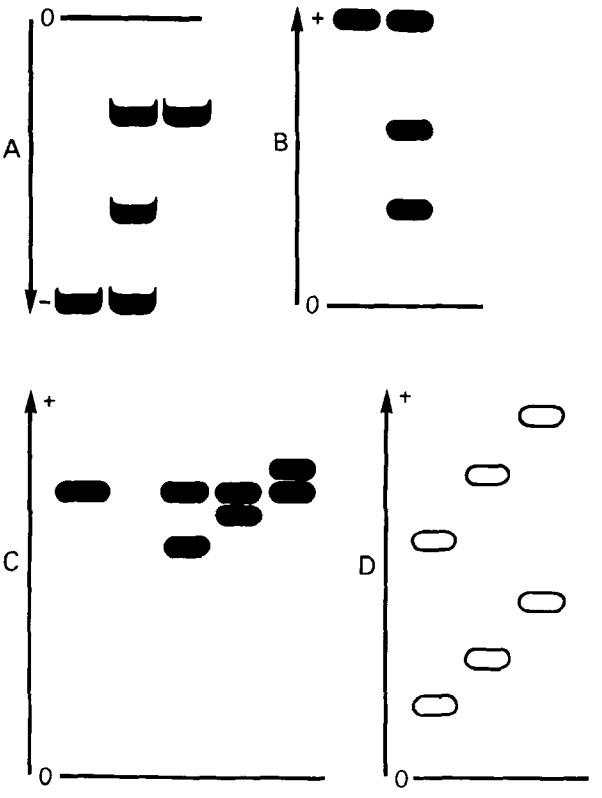


Fig. 1. Typically-encountered allozyme genotypes, illustrating all alleles found. (A) *Mdh-a* genotypes: $Mdh\text{-}a^2/^2$, $Mdh\text{-}a^1/^2$, $Mdh\text{-}a^1/^1$. (B) *Mdh-b*: $Mdh\text{-}b^2/^2$, $Mdh\text{-}b^1/^2$; no $Mdh\text{-}b^1/^1$ homozygotes were seen. (C) *Es*: $Es^3/\text{-}$, $Es^0/^0$, $Es^1/^3$, $Es^2/^3$, $Es^3/^4$. (D) *Amy*: $Amy^1/^4$, $Amy^2/^5$, $Amy^3/^6$; *Amy* gel patterns consisted of clear bands on a blue to green background.

The basis of allele frequency determinations can be understood with regard to the observed colony types at the *Mdh-a* locus:

		Male	
		$Mdh\text{-}a^1$	$Mdh\text{-}a^2$
	$Mdh\text{-}a^1/_1$	*a*	*b*
Queen	$Mdh\text{-}a^1/^2$	*c*	*d*
	$Mdh\text{-}a^2/^2$	*e*	*f*

unknown (either *b* or *e*): *u*

Note that *b* and *c* colonies have identical worker compositions: all are heterozygous. Such colonies can still be classified if the queen is captured, or if males from queen-laid eggs are taken with the colony (Crozier, 1974). Where all colonies in a sample have been unambiguously classified (there are no *u* colonies), gene frequencies can be determined by simple counting. Where *u* colonies occur, an iterative technique was employed involving successive partitioning of the *u* class on the basis of the ratios to one another of the unambiguously-classified colonies. The iteration procedure does not rely on there being Hardy-Weinberg proportions, and simulation showed that it is not biassed by departure from these proportions, except through non-random mating. No variance measure is currently available for estimates obtained through this procedure.

Es allele frequency estimates were made in two stages: the frequency of the null allele being estimated first. Where both colonies with all workers null (frequency g) and ones with both null and *Es*-positive workers (frequency h) occurred, the parental queen (x) and male (r) null frequencies were estimated by:

$$x = \frac{2g}{2g + h} \quad ; \quad r = \frac{g + h}{x(2 - x)}$$

Where only one colony type with null workers was found (all null), separate allele frequency estimates could not be made between the sexes, and the assumed common frequency (p), where K represents the null-containing colonies, was estimated by solving the equation:

$$K = 2p^2 - p^3 .$$

The colonies with no null phenotypes were then treated together and active allele frequencies estimated by iteration. These estimates were multiplied by $1 - x$ (for queens) and $1 - r$ (for males) to yield the final estimates for all alleles. Needless to say, no variance measure is currently available for these estimates.

Isozyme patterns for *A. fulva* and *treatae* indicated the same modes of inheritance occur in these species as in *rudis* for the four loci.

Large-scale colour- and isozyme variation

Considerable colour variation occurs between different populations of *rudis*. From New Jersey southwards a light brownish-red form occurs on the coastal plains between the Appalachians and the coast; this form will be termed the Coastal Plains (CP) form. In upper New York state, for example, and in the Appalachian Mountains further south, one finds darker brown to black ants, termed Montane (M) ants. This category will be shown to be itself heterogeneous. Dark ants of the *rudis* group also occur in Florida, although taxonomists differ as to whether these should be placed in '*rudis*' or not.

In Georgia, the Coastal Plains form is fixed for Es^0 except at some mountain localities and has intermediate frequencies (55%) of $Mdh\text{-}a^1$. Montane ants have low frequencies of Es^0, $Mdh\text{-}a^1$ rare or absent, and a rare allele, $Mdh\text{-}b^1$, at one locality. In this overall view, *Amy* allele frequencies do not differ greatly between *rudis* forms (but *fulva* is characterized by fixation for Amy^5), but finer-scale analysis below will demonstrate important patterns at this locus as well.

Thirty-eight samples from 30 populations at 16 localities are considered, as shown in Figure 2 and listed in Tables 1 and 2.

Table 1

Collection data. Words in *italics* are used to refer to sites in the text.

Sample number	Site	Collection date(s)
1	*Research Road* at Barnett Shoals Rd., Clarke Co., Ga.	March, April May, 1970
2	*Research Road* at Barnett Shoals Rd., Clarke Co., Ga.	May, June August, 1972
3	*Research Road* at Barnett Shoals Rd., Clarke Co., Ga.	April, 1973
4	*Research Road* at Barnett Shoals Rd., Clarke Co., Ga.	March, April, 1974
5, 38	*2.5 mi.* N. of Greensboro, *Greene* Co., Ga.	June, 1971
6	*2.5 mi.* N. of Greensboro, *Greene* Co., Ga.	April, 1974
7	*2.6 mi.* NE of Turnerville, Habersham Co., Ga.	August, 1970 April, May, 1971

Table 1 *(continued)*

Collection data. Words in *italics* are used to refer to sites in the text.

Sample number	Site	Collection date(s)
8, 21	*1.4 mi.* NNW of junction between highways US19 and US60, *Lumpkin* Co., Ga.	July, 1973
9, 22	*4.7 mi.* N. of *Turner's Corner*, junction of US19 and US129, Lumpkin Co., Ga.	June, July, 1973
10, 17, 23	*1.9 mi.* N. of *Turner's Corner*, Lumpkin Co., Ga.	May, 1973
11, 18, 25	*1.6 mi.* N. of *Turner's Corner*, Lumpkin Co., Ga.	May, June, 1973
12, 26	*1.6 mi.* N. of *Turner's Conner*, Lumpkin, Co., Ga.	June, 1974
13	*0.8 mi.* N. of *Turner's Corner*, Lumpkin Co., Ga.	July, 1973
14, 27	*0.8 mi.* N. of *Turner's Corner*, Lumpkin Co., Ga.	May, 1974
15, 31	*2.0 mi.* down road from kiosk, *Black Rock Mountain* State Park, Rabun Co., Ga.	July, 1974
16, 37	*2.5 mi.* NE. of Warrenton, *Warren* Co., Ga.	April, 1974
19	Bob Creek Road *1.75 mi.* from rte. 180, *Union Co.*, Ga.	May, 1972
20, 36	34th Street *1.3 mi.* N. of Archer Rd., *Alachua* Co., Fla.	September, 1973
24	*1.9 mi.* N. of *Turner's Corner*, Lumpkin Co., Ga.	June, 1974
28, 33	Near refreshment *kiosk*, *Black Rock Mountain* State Park, Rabun Co., Ga.	July, 1974
29, 34	*0.9 mi.* down road from kiosk, *Black Rock Mountain* State Park, Rabun Co., Ga.	June, July, 1974
30, 35	*1.1 mi.* down road from kiosk, *Black Rock Mountain* State Park, Rabun Co., Ga.	June, July, 1974
32	Rte. *17.1 mi.* S. of Towne Co. line *White* Co., Ga.	Summer, 1972

Comparison of observed and expected genotypic proportions

The expected numbers of heterozygote and homozygote queens were calculated using Levene's (1949) small-sample correction for all samples and loci lacking ambiguously-classified colonies. In only two cases, *Mdh-a* for Research Road in 1970 and 1974, were there more than five expected individuals in both classes so that a χ^2 could be reliably calculated. The 1974 sample did not deviate significantly from expectation, but, as reported previously (Crozier, 1973) the 1970 sample did ($P < 0.01$). There is a small overall bias towards heterozygosity in the data as a whole which is not shown in a χ^2 test (performed with lumped data as required), because of the loss of the information about the sign of the deviation, but when the normal deviate $\Sigma\chi/\sqrt{\text{d.f.}}$ is calculated (Simpson et al., 1960, p. 331), the heterozygote excess is significant ($P < 0.05$). It would be unwise to place much weight on this heterozygote excess, not only because the statistical evidence for it is not very strong, but also because biasses occurred in collecting the data that cannot be easily compensated for. The bias towards misclassification of heterozygote queens as homozygotes seems likely to be small (< 1%) if, as is apparently the case (Crozier, 1973), heterozygote queens have approximately 50% heterozygote worker offspring. Two sources of bias exist to increase the apparent frequency of queen heterozygotes. Firstly, there is a low rate of multiple-mating or, possibly, polygyny (multiple-queened colonies) in the Montane forms (five such possible colonies were found and excluded from the data reported here). A colony with two ovipositing queens of different genotype could 'mimic' one with a single heterozygote queen. However, this bias seem likely to be slight as the rate of occurrence of atypical colonies was very low considering the number of loci available for detection of the phenomenon. The second bias towards overestimating queen heterozygosity is more subtle but probably more serious: samples with, by chance, an excess of homozygote queens are more likely to have ambiguously-classified colonies and be excluded from the calculation of queen frequencies.

The extreme heterozygote excess found in the Research Road sample for 1970 was not seen in subsequent samples from that site. The apparent change might indicate a local shift in selection condi-

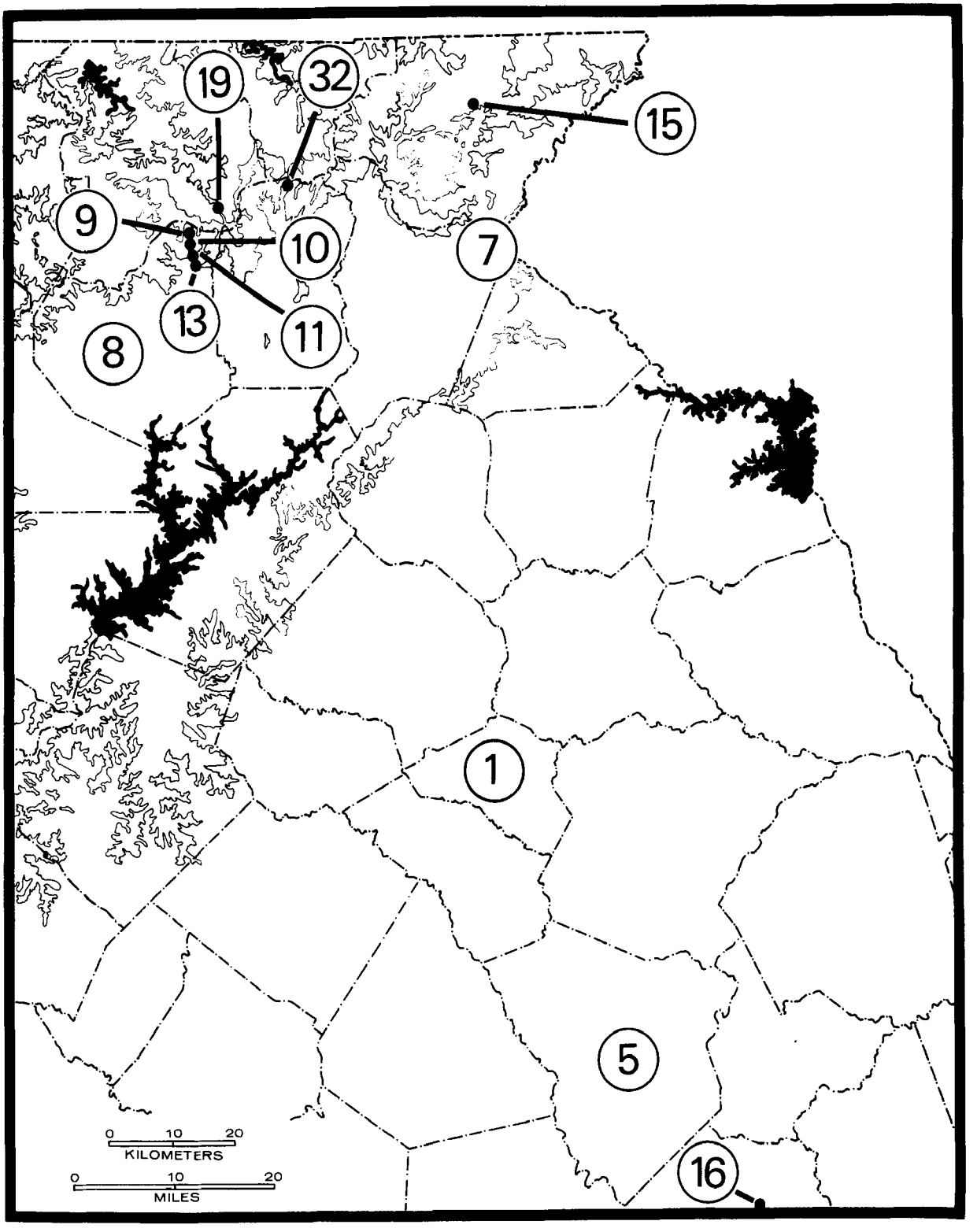


Fig. 2. Georgian localities for samples listed in Table 1. Where more than one sample was taken from a locality, only one sample number is shown in the figure. The scale of the figure does not permit separate indication of Black Rock Mountain localities. The contour interval is 1000 ft. (305 m).

Table 2

Gene frequency estimates in colonies of *Aphaenogaster 'rudis'*

N = number of colonies sampled (three genomes were sampled per colony); He = number of mother queen heterozygotes. True binomial 95% confidence limits (Hoel, 1962: p239) are given for the weighted averages for polymorphic samples with no ambiguously-classified colonies. References to *'rudis'* forms are CP, Coastal Plains; CP (20), Coastal Plains karyotyped with n = 20; Int, intermediate, unkaryotyped; M(?22), Montane, unkaryotyped but presumed to have n = 22; M(22), Montane, karyotyped with n = 22; M(18), 18-chromosome Montane form; Al. Alachua ? *rudis*. A rare allele, *Mdh-a*^{3}, occurred in sample 16.

Sample		*Mdh-a*		N	He	*Mdh-b*		N	He	*Es*					N	*Amy*				N	He
		1	2			1	2			0	1	2	3	4		1&2	3	4	5&6		
1 CP	♂	.73	.27																		
	♀	.50	.50																		
	Av	.58	.42	11	11																
	U	.99	.92																		
	L	.39	.25																		
2 CP	♂	.69	.31																		
	♀	.64	.36																		
	Av	.66	.34	72	34																
3 CP	♂	.50	.50																		
	♀	.69	.31																		
	Av	.63	.37	16	6																
4 CP	♂	.33	.67			0	1.0			1.0	0	0	0	0		.29	.21	.21	.29		
	♀	.60	.40			0	1.0			1.0	0	0	0	0		.25	.43	.07	.25		
	Av	.51	.49	15	10	0	1.0	15	0	1.0	0	0	0	0	15	.26	.36	.11	.27	14	10
	U	.99	.92																		
	L	.44	.26																		
5 CP	♂	.67	.33																		
	♀	.56	.44																		
	Av	.60	.40	14	6																
6 CP	♂	.91	.09			0	1.0			1.0	0	0	0	0		.27	.46	.18	.09		
	♀	.46	.54			0	1.0			1.0	0	0	0	0		.36	.32	.14	.18		
	Av	.61	.39	12	5	0	1.0	12	0	1.0	0	0	0	0	12	.33	.37	.15	.15	11	8
7 CP	♂	.67	.33																		
	♀	.56	.44																		
	Av	.60	.40	19	7																
8 CP + Int	♂	.75	.25			0	1.0			1.0	0	0	0	0		.25	0	.25	.5		
	♀	.75	.25			0	1.0			1.0	0	0	0	0		.375	.25	.375	0		
	Av	.75	.25	4	2	0	1.0	1	0	1.0	0	0	0	0	2	.33	.17	.33	.16	4	4
	U	.99	.66													.78	.52	.78	.52		
	L	.43	.05													.10	.02	.10	.02		
9 CP + Int	♂	1.0	0							1.0	0	0	0	0		0	.50	.50	0		
	♀	.50	.50							1.0	0	0	0	0		0	1.0	0	0		
	Av	.67	.33	2	2					1.0	0	0	0	0	2	0	.83	.17	0	2	1
	U	.99	.81																		
	L	.33	.22																		
10 CP	♂	0	1.0							0	.50	0	.50	0		0	1.0	0	0		
	♀	.25	.75							0	0	0	1.0	0		0	.25	0	.75		
	Av	.17	.83	2	1					0	.17	0	.83	0	2	0	.50	0	.50	2	1
	U	.64	1.0													.46	.94	.46	.94		
	L	.004	.36													0	.12	0	.12		

Table 2 (contd)

Sample		*Mdh-a*		N	He	*Mdh-b*		N	He	*Es*					N	*Amy*				N	He
		1	2			1	2			0	1	2	3	4		1&2	3	4	5&6		
11 CP	♂	.71	.29							.98	0	0	.02	0		.18	.29	.39	.14		
	♀	.79	.21							.76	0	0	.24	0		.21	.43	.18	.18		
	Av	.76	.24	18	6					.83	0	0	.17	0	14	.20	.38	.25	.17	14	9
12 CP (20)	♂	.60	.40			0	1.0			.94	0	0	.06	0		.22	.14	.21	.43		
	♀	.63	.37			0	1.0			.87	0	0	.13	0		.28	.50	.11	.11		
	Av	.62	.38	15	9	0	1.0	17	0	.89	0	0	.11	0	14	.26	.38	.14	.22	14	7
13 CP	♂	.64	.36			0	1.0			.93	0	0	.07	0		.07	.77	.08	.08		
	♀	.61	.39			0	1.0			.93	0	0	.07	0		.08	.42	.35	.15		
	Av	.62	.30	14	7	0	1.0	1	0	.93	0	0	.07	0	14	.08	.54	.26	.13	13	10
	U															.25	.98	.71	.40		
	L															.02	.37	.13	.04		
14 CP (20)	♂	.50	.50			0	1.0			.94	0	0	.06	0		.11	.56	.22	.11		
	♀	.75	.25			0	1.0			.80	0	0	.20	0		.33	.50	0	.17		
	Av	.67	.33	10	5	0	1.0	10	0	.85	0	0	.15	0	10	.26	.52	.07	.15	9	6
15 CP (20)	♂	.50	.50			0	1.0			1.0	0	0	0	0		.50	.50	0	0		
	♀	.50	.50			0	1.0			1.0	0	0	0	0		.25	.50	.25	0		
	Av	.50	.50	2	0	0	1.0	2	0	1.0	0	0	0	0	2	.33	.50	.17	0	2	2
	U															.81	.94	.64	.46		
	L															.04	.12	.004	0		
16 CP	♂	.45	.55			0	.90			1.0	0	0	0	0		.12	.55	0	.33		
	♀	.60	.40			0	1.0			.90	0	0	.10	0		.33	.45	.22	0		
	Av	.55	.45	10	5	0	.97	10	0	.93	0	0	.07	0	10	.26	.48	.15	.11	9	7
17 Int.	♂	0	1.0							.50	0	0	.50	0		0	.50	0	.50		
	♀	.25	.75							.50	0	0	.50	0		0	.75	.25	0		
	Av	.17	.83	2	1					.50	0	0	.50	0	2	0	.66	.17	.17	2	1
	U	.64	1.0													.46	.99	.64	.64		
	L	.004	.36													0	.22	.004	.004		
18 Int.	♂	.60	.40							.67	0	0	.33	0		0	.60	.20	.20		
	♀	.50	.50							.67	0	0	.33	0		.10	.50	.20	.20		
	Av	.53	.47	5	1					.67	0	0	.33	0	5	.07	.53	.20	.20	5	4
	U	.96	.92													.32	.96	.56	.56		
	L	.27	.21													.002	.27	.04	.04		
19 Int.	♂	0	1.0							0	.09	.27	.64	0		0	.25	.12	.63		
	♀	.04	.96							0	.05	.09	.86	0		0	.06	.44	.50		
	Av	.02	.98	14	1					0	.06	.15	.79	0	11	0	.12	.34	.54	8	7
	U	.16	1.0													.14	.39	.80	.89		
	L	.001	.87													0	.03	.16	.33		
20 Al.	♂	.25	.75			0	1.0			0	.33	.17	.50	0		.13	.50	0	.37		
	♀	.25	.75			0	1.0			0	.50	.17	.33	0		.24	.25	.13	.38		
	Av	.25	.75	4	2	0	1.0	4	0	0	.44	.17	.39	0	3	.21	.33	.08	.37	4	3
	U	.66	.99																		
	L	.05	.43																		
21 M (? 22)	♂	.20	.80			0	1.0			.28	.06	.11	.55	0		0	.27	.38	.35		
	♀	.27	.73			0	1.0			.28	.11	0	.61	0		.17	.20	.29	.34		
	Av	.25	.75	16	3	0	1.0	1	0	.28	.09	.04	.59	0	15	.11	.22	.32	.35	15	12
22 M (?22)	♂	.07	.93			0	1.0			0	.07	0	.93	0		0	.36	.43	.21		
	♀	.04	.96			0	1.0			0	.07	.04	.89	0		.07	.21	.29	.43		
	Av	.05	.95	14	1	0	1.0	2	0	0	.07	.02	.91	0	14	.04	.26	.34	.36	14	8
	U	.18	1.0																		
	L	.01	.84																		

Tabel 2 (contd)

Sample	*Mdh-a*		N	He	*Mdh-b*		N	HE	*Es*					N	*Amy*				N	He
	1	2			1	2			0	1	2	3	4		1&2	3	4	5&6		
23 M (?22)	♂ .07	.93							0	.07	0	.93	0		.28	.07	.29	.36		
	♀ .07	.93							0	.07	0	.93	0		.14	.18	.46	.22		
	Av .07	.93	14	2					0	.07	0	.93	0	14	.20	.14	.40	.26	14	10
	U .24	.10																		
	L .01	.81																		
24 M (22)	♂ 0	1.0			0	1.0			.27	0	0	.73	0		.21	.36	.29	.14		
	♀ 0	1.0			0	1.0			.27	.05	0	.68	0		.21	.14	.36	.29		
	Av 0	1.0	8	0	0	1.0	8	0	.27	.03	0	.70	0	8	.21	.22	.33	.24	7	6
25 M (?22)	♂ 0	1.0							.39	0	0	.61	0		0	.50	.31	.19		
	♀ 0	1.0							.39	0	0	.61	0		.12	.38	.31	.19		
	Av 0	1.0	8	0					.39	0	0	.61	0	8	.08	.42	.31	.19	8	6
26 M(22)	♂ .50	.50			0	1.0			1.0	0	0	0	0		.50	0	0	.50		
	♀ 1.0	0			0	1.0			1.0	0	0	0	0		0	.50	.25	.25		
	Av .83	.17	2	0	0	1.0	2	0	1.0	0	0	0	0	2	.17	.33	.17	.33	2	2
	U														.64	.81	.64	.81		
	L														.004	.04	.004	.04		
27 M (22)	♂ 0	1.0			0	1.0			0	0	0	1.0	0		0	.50	0	.50		
	♀ 0	1.0			0	1.0			0	0	0	1.0	0		0	.50	0	.50		
	Av 0	1.0	2	0	0	1.0	2	0	0	0	0	1.0	0	2	0	.50	0	.50	1	0
28 M (22)	♂ 0	1.0			0	1.0			0	0	0	.86	.14		0	0	.50	.50		
	♀ 0	1.0			0	1.0			0	0	0	1.0	0		.07	.14	.36	.43		
	Av 0	1.0	7	0	0	1.0	7	0	0	0	0	.95	.05	7	.05	.10	.40	.45		
29 M (22)	♂ 0	1.0			0	1.0			.47	0	0	.53	0		.17	0	0	.83		
	♀ 0	1.0			0	1.0			.47	0	0	.53	0		.33	.33	0	.34		
	Av 0	1.0	3	0	0	1.0	3	0	.47	0	0	.53	0	3	.28	.22	0	.50	3	2
30 M (22)	♂ 0	1.0			0	1.0			.29	0	0	.71	0		.07	.14	.43	.36		
	♀ 0	1.0			0	1.0			.29	0	0	.71	0		.04	.14	.29	.53		
	Av 0	1.0	14	0	0	1.0	14	0	.29	0	0	.71	0	14	.05	.14	.33	.48	14	8
31 M (22)	♂ 0	1.0			0	1.0			.35	0	0	.60	.05		.07	0	.36	.57		
	♀ 0	1.0			0	1.0			.35	0	0	.65	0		.04	.07	.18	.71		
	Av 0	1.0	15	0	0	1.0	15	0	.35	0	0	.63	.02	15	.05	.05	.24	.66	14	5
32 M (?22)	♂ .20	.20																		
	♀ 0	1.0																		
	Av .07	.93	5	0																
33 M (18)	♂ .67	.33			0	1.0			0	0	.17	.83	0		0	0	1.0	0		
	♀ .75	.25			.08	.92			0	0	.08	.92	0		0	0	1.0	0		
	Av .72	.28	6	3	.06	.94	6	1	0	0	.11	.89	0	6	0	0	1.0	0	6	0
	U				.27	1.0														
	L				.001	.73														
34 M (18)	♂ .67	.33			.33	.67			0	0	.33	.67	0		0	0	1.0	0		
	♂ .33	.67			0	1.0			0	0	.17	.83	0		0	0	1.0	0		
	Av .44	.56	3	2	.11	.89	2	0	0	0	.22	.78	0	3	0	0	1.0	0	3	0
	U .90	.96																		
	L .14	.21																		
35 M (18)	♂ .21	.79			0	1.0			0	0	.20	.80	0		0	0	1.0	0		
	♀ .69	.31			.10	.90			0	0	.20	.80	0		0	0	1.0	0		
	Av .53	.47	5	1	.07	.93	5	1	0	0	.20	.80	0	5	0	0	1.0	0	4	0
	U				.32	.10														
	L				.002	.68														

Table 2 (contd)

Sample		*Mdh-a*		N	He	*Mdh-b*		N	He	*Es*					N	*Amy*				N	He
		1	2			1	2			0	1	2	3	4		1&2	3	4	5&6		
36 *fulva*	♂	0	1.0			0	1.0			0	0	0	1.0	0		0	0	0	1.0		
	♀	0	1.0			0	1.0			0	.10	0	.90	0		0	0	0	1.0		
	Av	0	1.0	5	0	0	1.0	5	0	0	.07	0	.93	0	5	0	0	0	1.0	5	0
37 *fulva*	♂	0	1.0			0	1.0			.92	0	0	.08	0		0	0	0	1.0		
	♀	0	1.0			0	1.0			.75	0	.04	.21	0		0	0	0	1.0		
	Av	0	1.0	12	0	0	1.0	12	0	.80	0	.03	.17	0	12	0	0	0	1.0	10	0
38 *treatae*	♂	0	1.0			0	1.0			0	0	1.0	0	0		0	.50	.50	0		
	♀	0	1.0			0	1.0			0	0	1.0	0	0		.25	.75	0	0		
	Av	0	1.0	2	0	0	1.0	2	0	0	0	1.0	0	0	2	.17	.66	.17	0	2	1
	U															.64	.99	.64	.46		
	L															.004	.22	.004	0		

tions, which was one suggestion made previously (Crozier, 1973), or indicate that the departure from Hardy-Weinberg proportions, although statistically highly significant ($P < .01$), could be spurious and due to sampling error.

Gametic phase imbalance at one site, and its explanation

In 1973 I found a site (Turner's Corner, Lumpkin Co.) in the foothills of the Appalachians where the change from the great majority of colonies being of the Coastal Plains form to where the Montane phenotype predominates takes place over a distance of just over one kilometre (Fig. 3). Colonies were classified, on the basis of worker colour, as Coastal Plains form, Montane form, or 'intermediate' – colonies that could not unequivocally be allocated to either form. This classification was made independently of the isozyme information.

The male genotypes of the unambiguously classified colonies showed a strong association between alleles at the *Es* and *Mdh-a* loci:

	Es^0	Es^3	
$Mdh\text{-}a^1$	12	0	P = 0.00004 (two-tailed Fisher's exact test)
$Mdh\text{-}a^2$	1	9	

However, the association beteen alleles is completely explainable by association at this site between colour form and isozyme genotype.

	Montane	Intermediate	Coastal Plains
Es^0	1	3	10
Es^3	7	2	0
$Mdh\text{-}a^1$	0	3	10
$Mdh\text{-}a^2$	8	2	0

Figures 4, 5, and 6 show the allele frequencies at the different sites at this locality in 1973.

1974 analysis of the Turner's Corner genetic boundary

The most likely explanation of the observed associations between alleles and colour forms seemed to be that the colour forms are in fact sibling species. However, a noted ant taxonomist, W.L. Brown, advised that the great morphological similarity (apart from colour) of these ants indicated instead one species with multiple stepped clines in allozyme frequencies. Accordingly, in 1974, as many colonies as possible at mountain localities were karyotyped as well as electrophoresed, and scattered colonies karyotyped elsewhere.

Chromosomally, there is no intergradation at Turner's Corner between the Coastal Plains form with n = 20 and the Montane form with n = 22; no intermediate numbers were found (Fig. 7). The presence of Es^0 in Montane and Es^3 in Coastal Plains samples in this contact zone suggest that some gene flow occurs, but the overall pattern of allozyme frequencies, and the lack of chromosomal intermediates, show that it must be very restricted.

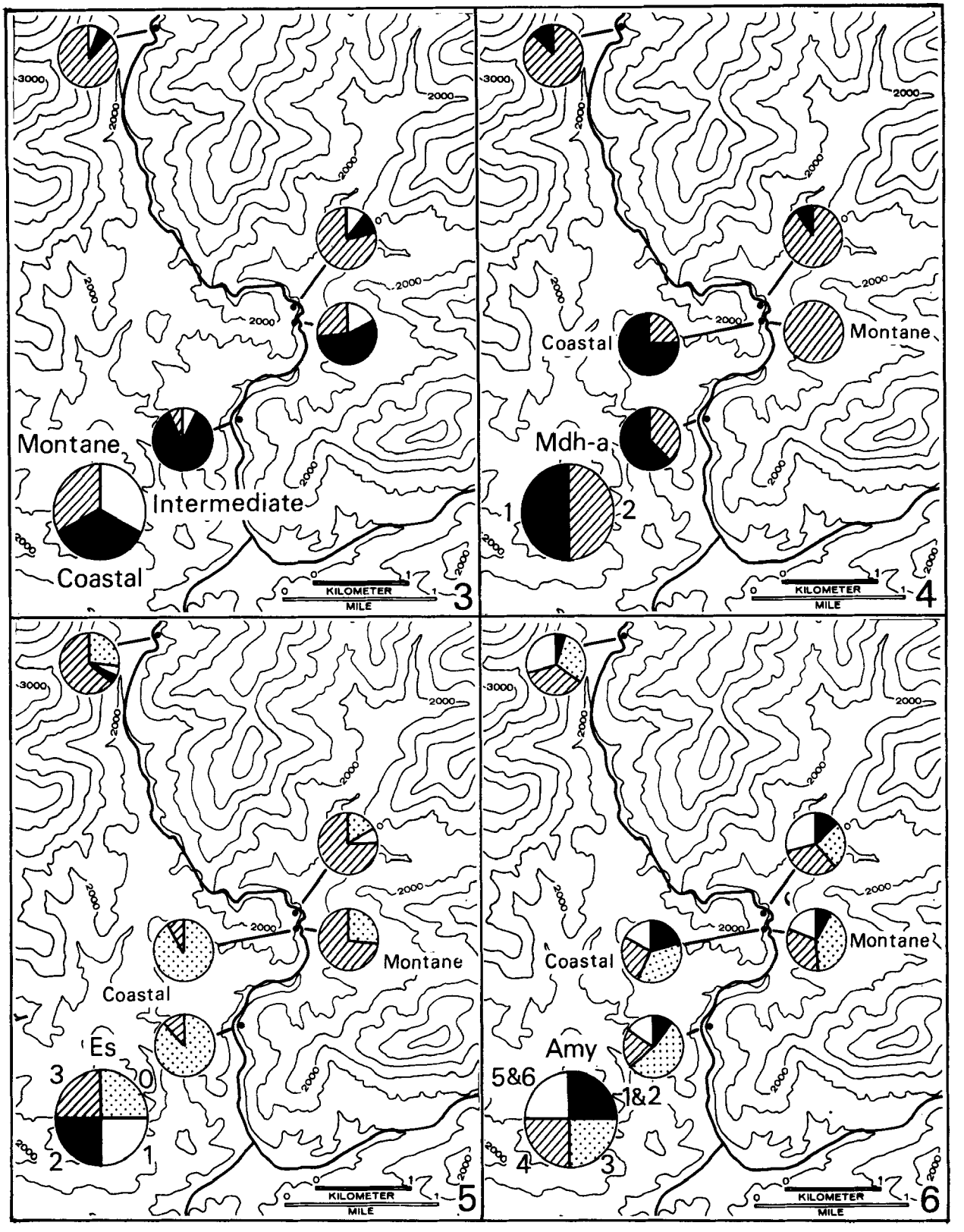
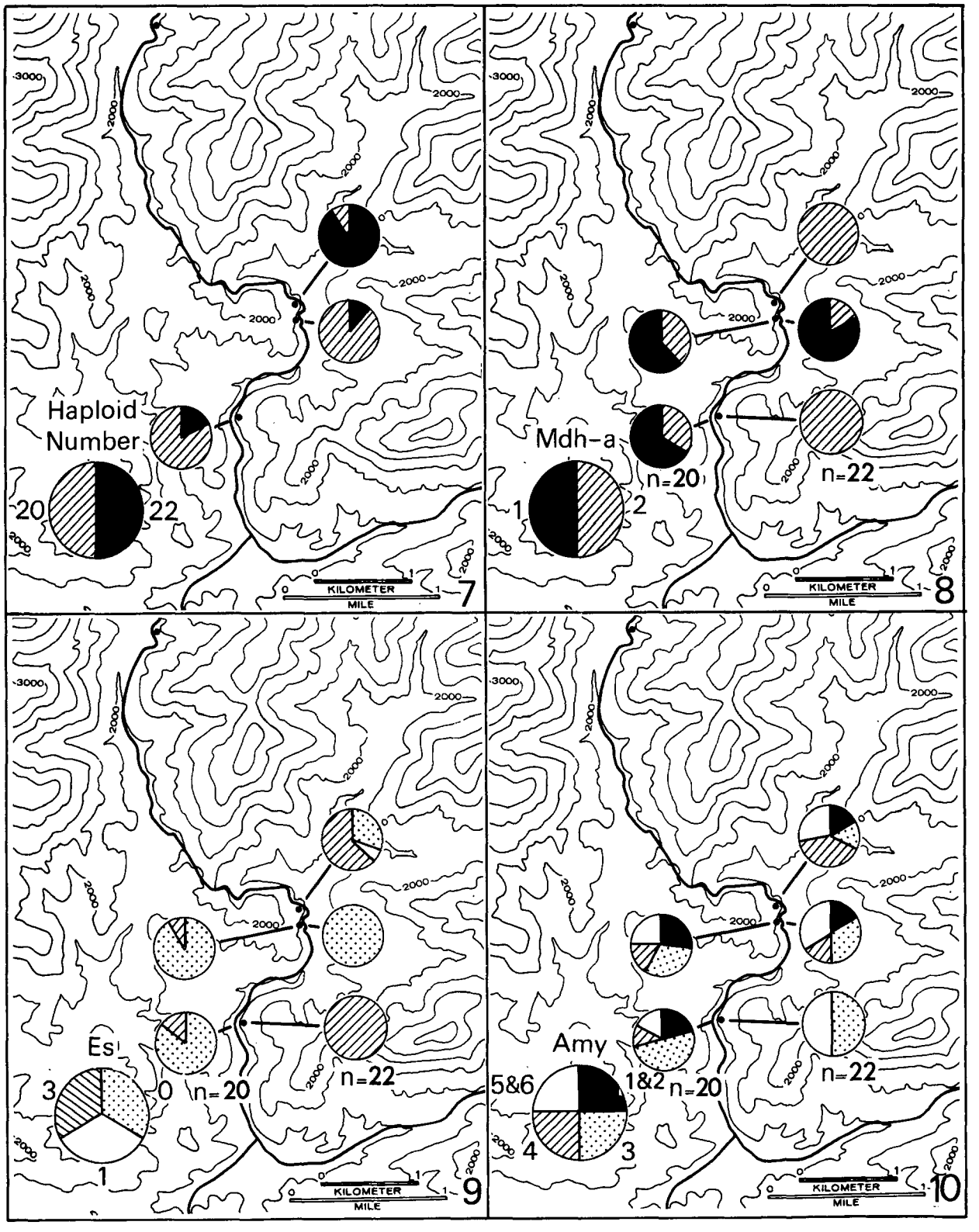


Fig. 3-6. Turner's Corner, 1973 (junction between U.S. highways 19 and 129). Contours in this and subsequent maps are given in feet (one foot = 0.3 m): (3) frequencies of morphological forms of *A. 'rudis'*; – (4-6) allele frequencies of *Mdh-a* (4), *Es* (5) and *Amy* (6).



Figs. 7-10. Turner's Corner, 1974: (7) chromosome numbers of *A. 'rudis'* populations; – (8-10) allele frequencies of *Mdh-a* (8), *Es* (9) and *Amy* (10).

No sorting of colonies into Montane, Coastal Plains, and 'intermediate' forms was performed for 1974, as this process is time-consuming and difficult. Cursory examination of the specimens indicated that the Montane and Coastal Plains forms correspond to the 22- and 20-chromosome cytotypes respectively. At the site of maximum overlap (1.6 mi.), comparison of the *Mdh-a* and *Es* male frequencies using Fisher's Exact Test showed no significant difference between the 1973 Coastal Plains form and the 1974 20-chromosome colonies for either locus, but $P < 0.01$ for both loci for the , 973 Montane-1974 20-chromosomes comparison. Too few 22-chromosome colonies were found for such comparisons.

The allele frequency patterns for 1974 (Figs. 8-10) are compatible with those for 1973 (Figs. 4-6).

Genetic differentiation at Black Rock Mountain

Two haploid numbers were found among Montane ants at Black Rock Mountain State Park: 22 and 18 (Fig. 11). The 18-chromosome ants were most numerous at the topmost site, declining in frequency with decreasing altitude. No cytological evidence of hybridization between these two cytotypes was found: there were no colonies with intermediate numbers, save for two referable to the Coastal Plains form (more detailed karyotype analysis will be presented below).

The 18- and 22-chromosome colonies on Black Rock Mountain tended to nest in different microhabitats, especially at the highest site (next to the refreshment kiosk):

	in logs	under stones
18-chromosomes	6	0
22-chromosomes	0	7

The above result differs significantly from random assortment both by the Intra-Ocular Traumatic Test (Edwards et al., 1963) and by Fisher's Exact Test ($P = 0.0012$).

The 18- and 22-chromosome cytotypes differed in allozyme constitution (Figs. 12-15; Tab. 2). The rare allele $Mdh\text{-}b^1$ is restricted to the 18-chromosome form, which is fixed for Amy^4 whereas all the other *Amy* alleles except Amy^1 occur in the 22-chromosome form. The sole 18-chromosome colony taken at the lowest site, however, had an $Amy^4/^5$ heterozygote queen. The 22-chromosome form is fixed for $Mdh\text{-}a^2$, but the 18-chromosome form has $Mdh\text{-}a^1$ frequencies of 0.44 or higher. Es^2 is restricted to the 18-chromosome form and Es^0 and Es^4 to the other, with Es^3 being the commonest allele in both.

Although the 18- and 22-chromosome form samples differed significantly for allele frequencies among unambiguously classified colony males at the kiosk site for both *Mdh-a* and *Amy* ($P < 0.05$, Fisher's Exact Test), sample sizes were not sufficient to demonstrate non-random association between alleles at these loci for the total pooled data. Without the chromosomal evidence, it therefore would be extremely unlikely that the existence of two forms could be detected.

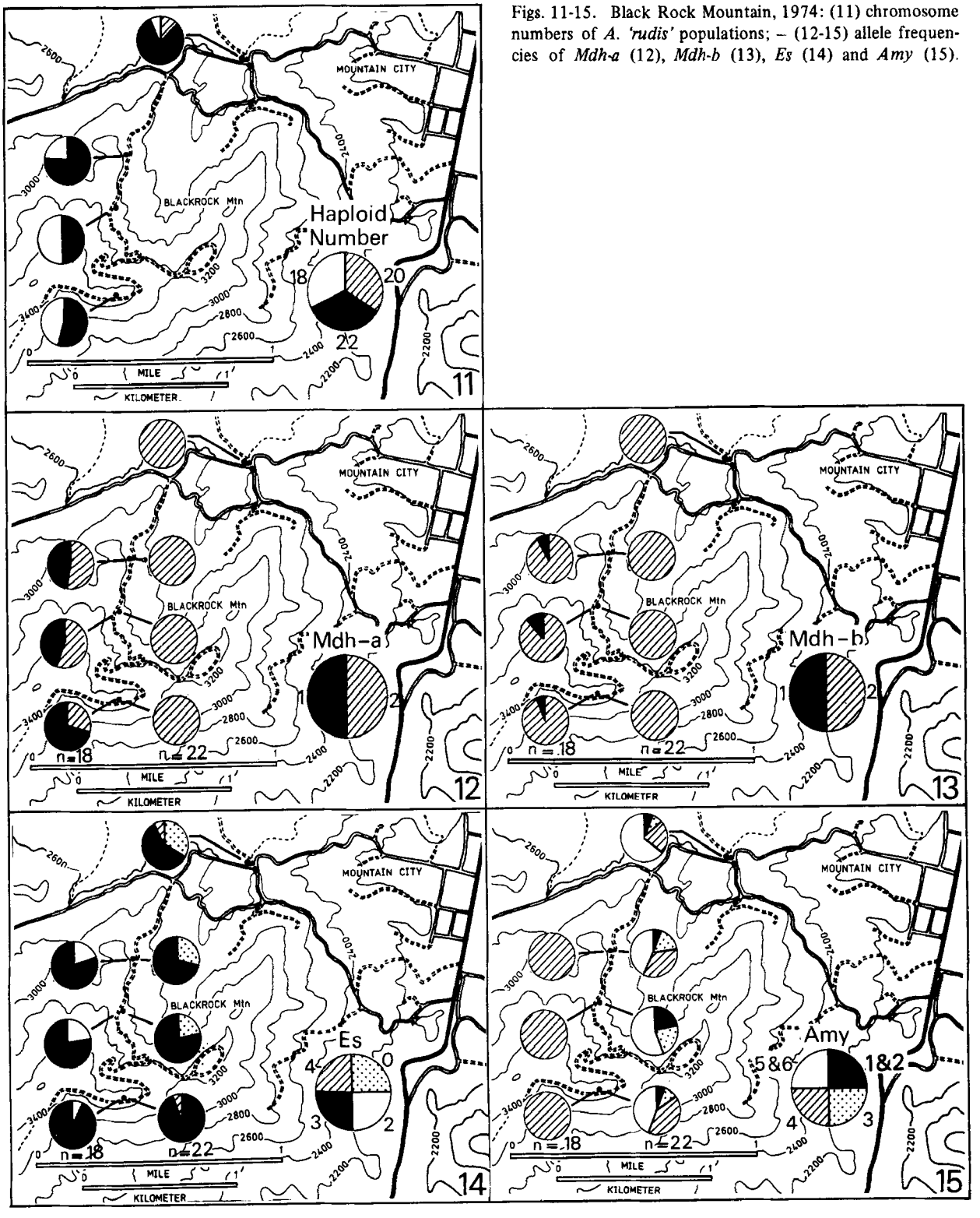
The taxonomist W.L. Brown selected ants from the 22-chromosome colony as lighter than those from an 18-chromosome colony for six pairs of such colonies presented to him. This colour difference was later confirmed by another prominent ant taxonomist, R.W. Taylor. The colour difference is, however, extremely slight and would neither be useful as a key character nor would it be found to delimit taxa in the absence of the chromosomal data. The 18-chromosome ants are somewhat variable morphologically, with some worker specimens in some colonies showing weak development of a character (a sharp longitudinally concave dorsal projection from the mesonotum) that is strongly developed in and diagnostic of *Aphaenogaster fulva*.

Allozyme frequencies in other species

Two colonies of *Aphaenogaster treatae* were fixed for $Mdh\text{-}a^2$, $Mdh\text{-}b^2$ and Es^2, but were polymorphic for *Amy*.

A sample of four colonies of dark ants of the *rudis* group from Alachua Co., Florida, was fixed for $Mdh\text{-}b^2$ but polymorphic at the other loci, lacking Es^0. The divergent karyotype of these ants, discussed below, suggests that this sample represents yet another 'form' in the '*rudis*' group.

Two samples of *fulva* were fixed for $Mdh\text{-}a^2$, $Mdh\text{-}b^2$, and Amy^5 but were polymorphic for *Es* (Tab. 2). Allelic composition at the *Es* locus paralleled that of sympatric *rudis*-group populations, with Es^0 present in Warren County *fulva*, as in sympatric 20-chromosome *rudis*, but absent in Alachua County *fulva*, as in the *rudis*-group sample from that locality.



Figs. 11-15. Black Rock Mountain, 1974: (11) chromosome numbers of *A. 'rudis'* populations; – (12-15) allele frequencies of *Mdh-a* (12), *Mdh-b* (13), *Es* (14) and *Amy* (15).

Karyotype analysis

Karyotype evolution in North American *Aphaenogaster* has been rapid and taken place through both Robertsonian and non-Robertsonian changes in chromosome number (Crozier, 1969, 1975). Relationships between the various karyotypes are complex, but the chromosomal evidence is extremely important in determining the distinctness of various taxa from each other.

The Coastal Plains form typically has n = 20 with a karyotype, characterised by two large metacentrics (Fig. 16), which is also found in this form in New Jersey (Crozier, 1969, 1975). Non-Robertsonian polymorphism for chromosome number was found in Greene County preparations, with diploid numbers of 41 and 42 found (Fig. 16). This polymorphism is reminiscent of the numerical polymorphism of one upper New York State *'rudis'* form (Crozier, 1969, 1975).

The 22-chromosome Montane karyotype (Fig. 17A) could possibly be derived from the 20-chromosome Coastal Plains karyotype through two Robertsonian changes, but this suggestion remains highly tentative in the absence of sufficient data on the smaller chromosomes.

Fulva and 18-chromosome *'rudis'* have essentially the same karyotype (Fig. 17B, C), which is resembled most among the others by that of an Alexander Springs, Florida, *rudis*-group (?*'miamiana'*) colony (Fig. 17D). These karyotypes cannot be easily derived from any other.

Another divergent karyotype is that of dark *'rudis'* from Alachua County, Florida (Fig. 18A). This 22-chromosome karyotype differs markedly from that of the Montane 22-chromosome form in the possession, among other things, of one distinctive large metacentric. The Alachua *'rudis'* karyotype somewhat resembles that of *A. lamellidens* (Fig. 18B; Warren County) in the possession of one large distinctive

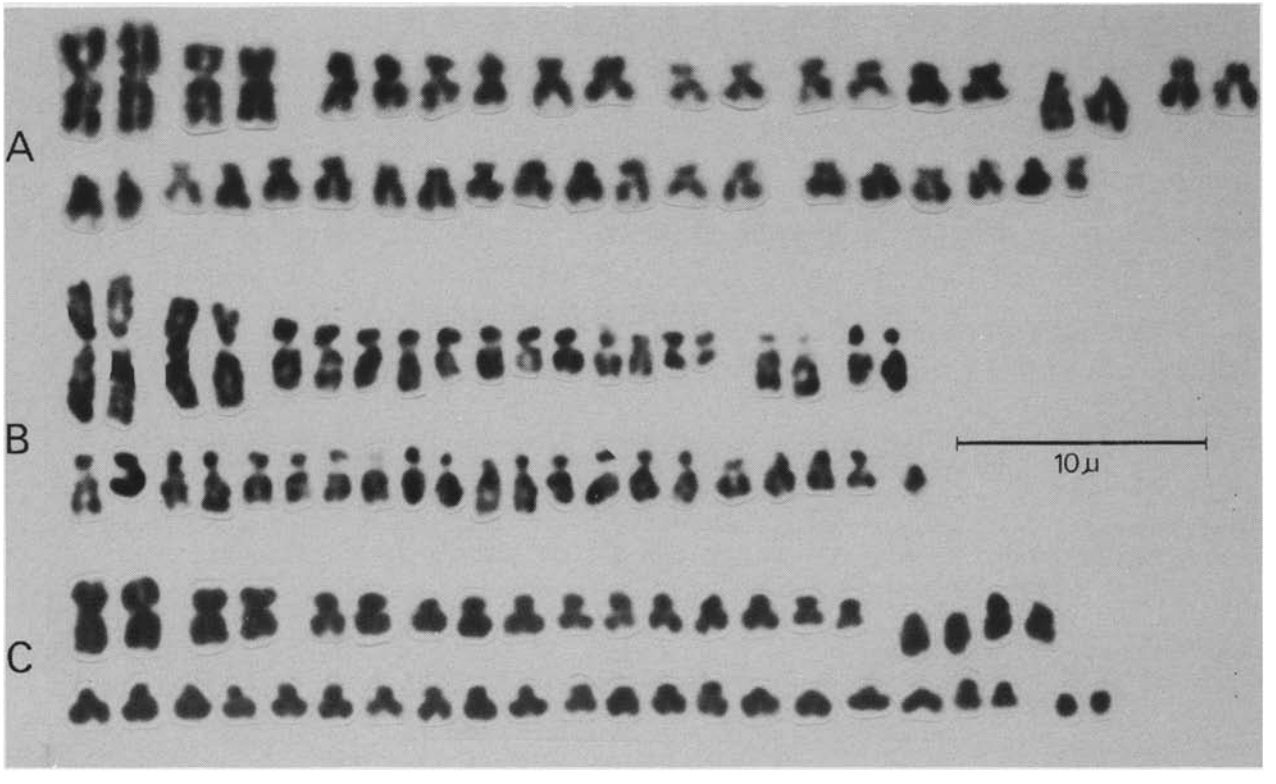


Fig. 16. Karyotypes of the Coastal Plains *Aphaenogaster 'rudis'* species: (A) normally-encountered diploid (2n=40) karyotype; – (B) and (C) karyotypes with 41 (B) and 42 (C) chromosomes found in the Greene County, Georgia population. The Greene Co. polymorphism is non-Robertsonian in nature, possibly being due to supernumerary chromosomes as suggested by the arrangement shown.

Fig. 17. Karyotypes of (A) 22-chromosome Montane *Aphaenogaster 'rudis'* (2n=44); – (B) 18-chromosome Montane *A. 'rudis'* (n=18); – (C) A. *fulva* (2n=36); – (D) *A. 'miamiana'* (2n=36).

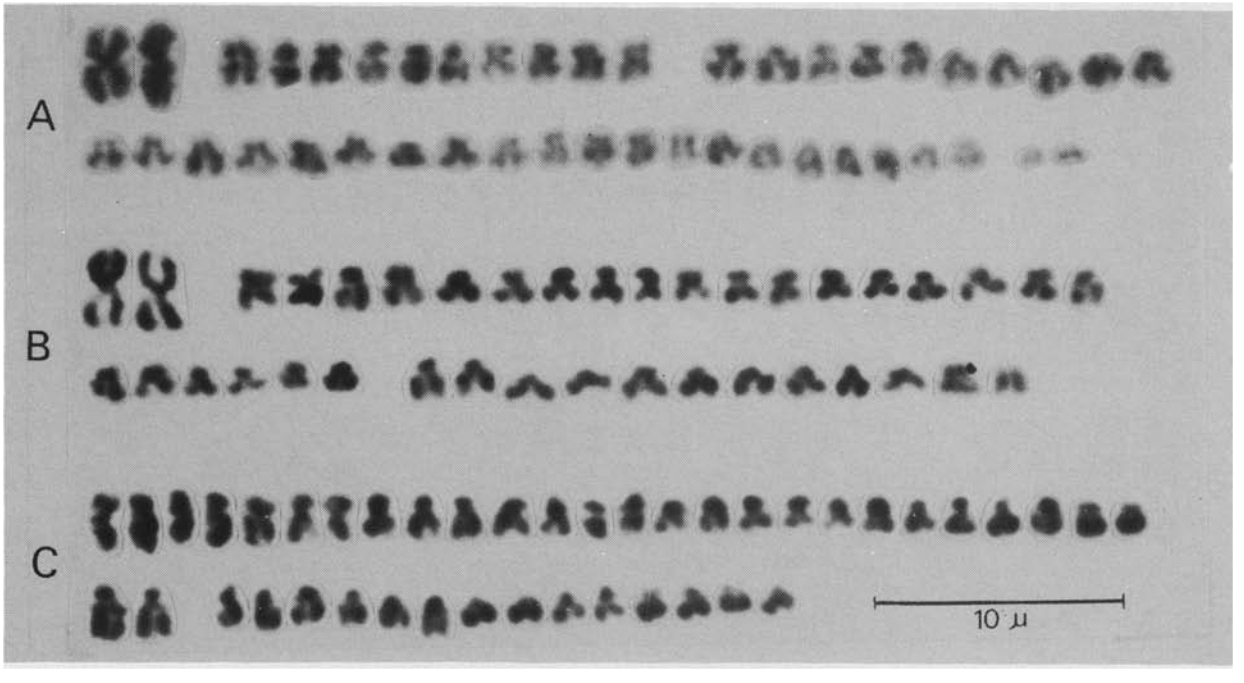


Fig. 18. Karyotypes of (A) *Aphaenogaster treatae* (2n=42); – (B) *A. lamellidens* (2n=38); – (C) *A. 'rudis'* from Alachua Co., Fla, (2n=44).

Table 3

Minimum genetic distances (D_m) between samples scored at four loci (upper-right section) and corresponding 95% confidence limits (lower-left section). These distance values are not true absolute values (see text). D_m values significantly differing from zero are shown in *italics*. Abbreviations are as for Tables 1 and 2. To save space, all values have been multiplied by 100.

Sample	4	6	8	12	13	14	15	16	20	21	22	24	26	27	28	29	30	31	33	34	35	36	37	38
4 CP	0	*1*	*3*	*7*	2	*2*	1	1	19	14	30	20	3	33	33	*15*	*20*	*21*	*37*	*34*	*34*	*39*	*17*	34
6 CP	.5	0	*1*	.4	1	*1*	*1*	*4*	21	*16*	*34*	*23*	*2*	*38*	*37*	*18*	*24*	*25*	*35*	*34*	*34*	*45*	*23*	36
8 CP+Int.	2	1	0	*2*	3	3	*4*	*3*	*25*	*18*	*37*	*27*	1	*45*	*40*	*24*	*27*	*21*	*31*	*29*	30	*49*	27	*43*
12 CP (20)	5	.5	1	0	1	4	*2*	*4*	17	*12*	*27*	*20*	*2*	*32*	*31*	*16*	*20*	*24*	*30*	*30*	*29*	*38*	*20*	*34*
13 CP	2	2	4	2	0	1	*2*	1	20	*14*	*30*	*22*	*2*	*34*	*34*	*19*	*22*	*24*	*30*	*30*	*29*	*44*	*24*	34
14 CP (20)	1	1	4	.5	1	0	*2*	*1*	18	*14*	*29*	*21*	*2*	*32*	*33*	*18*	*22*	*24*	*32*	*32*	*31*	*41*	*25*	*34*
15 CP (20)	2	1	3	1	2	1	0	*4*	21	*16*	32	*21*	*5*	*36*	*36*	*18*	*23*	*26*	*37*	*34*	*34*	*47*	24	32
16 CP	1	.3	3	.4	1	.4	.3	0	18	*13*	*28*	*19*	*3*	*32*	*33*	*16*	*20*	*22*	*33*	*31*	*31*	*42*	*22*	32
20 Al	26	26	25	20	21	18	25	22	0	4	*8*	*7*	26	10	*11*	8	*8*	*9*	*25*	20	*21*	*14*	*20*	18
21 M(?22)	16	15	16	11	12	9	15	12	5	0	3	2	*20*	*7*	*5*	*4*	2	3	*16*	11	*12*	*11*	*15*	23
22 M(?22)	34	33	34	26	28	23	33	28	8	3	0	2	*39*	3	6	*7*	3	*4*	*19*	*13*	*14*	8	22	27
24 M(22)	19	20	24	17	17	17	18	16	6	2	2	0	*31*	*6*	*3*	3	1	3	*22*	14	16	11	*17*	23
26 M(22)	4	2	2	2	2	14	4	3	26	18	36	28	0	*44*	*43*	*25*	*29*	*30*	*35*	*37*	*35*	*48*	*26*	*45*
27 M(22)	36	35	34	28	31	26	35	30	11	4	3	6	39	0	4	7	*6*	*7*	*32*	25	27	6	23	29
28 M(22)	34	33	35	26	28	23	33	28	9	4	6	3	37	6	0	*8*	3	2	*20*	*13*	*15*	6	22	31
29 M(22)	12	14	19	13	13	15	10	10	8	3	7	3	25	8	8	0	3	2	*35*	*27*	*29*	*10*	*8*	25
30 M(22)	15	15	22	14	14	15	14	12	6	2	3	1	26	5	4	3	0	*6*	*24*	*16*	*16*	*18*	7	26
31 M(22)	14	15	13	13	13	15	15	12	7	3	4	3	25	6	4	3	10	0	*28*	*20*	*22*	*5*	7	*29*
33 M(18)	34	34	28	30	30	29	34	30	19	11	18	19	34	30	20	25	19	21	0	2	1	*38*	*53*	*47*
34 M(18)	32	31	26	27	27	26	32	28	20	11	11	10	29	27	10	23	12	17	3	0	3	31	*43*	*35*
35 M(18)	33	32	31	27	28	27	33	29	20	11	12	12	30	27	12	23	12	18	1	3	0	33	*45*	*38*
36 *fulva*	31	31	31	24	27	23	34	27	11	10	12	15	33	10	9	9	13	5	38	37	37	0	15	*42*
37 *fulva*	14	20	23	18	22	23	25	21	16	11	23	16	25	25	24	7	8	7	32	34	34	24	0	*39*
38 *treatae*	36	36	36	33	35	33	37	35	19	25	34	30	38	38	36	28	28	29	27	23	23	39	36	0

metacentric and the overall appearance of the chromosome set, but the latter has n = 19. Adding finally to the complexity is the *treatae* karyotype with 21 pairs of chromosomes (Fig. 18C; Black Rock Mountain). The *treatae* karyotype is again different from all the others although, considering the occurrence of non-Robertsonian numerical polymorphism in two '*rudis*' forms, it does resemble the various 18-chromosome karyotypes.

The 18-chromosome karyotypes resemble that of the darker of two forms found around Ithaca, upper New York State (Crozier, 1969, 1975) although a number of pericentric rearrangements have occurred between the two. The karyotype of the other Ithaca form is not readily related to any considered here.

Genetic distances within and between taxa

Nei's (1972; Nei & Roychoudhury, 1974) D_m was used to estimate differentiation between populations according to the isozyme loci, because the variance estimate of this measure is relatively insensitive to sample size effects. The comparisons for the 24 samples for which all four loci were scored are given in Table 3; D_m are given in the upper right portion of the table, and the corresponding 95% confidence limits in the lower left section. Inspection of Table 3 shows that intra-taxon variation is much less than inter-taxon variation. Average values for D_m for within- and between-taxon comparisons are given in Table 4; the smallest samples were disregarded in

Table 4

Mean minimum genetic distances and 95% confidence limits between and within taxa computed from Table 3. Samples 8, 15, 20, 26, 27, 29 and 34 were excluded from these calculations because of possible sample size effects. Only one intra-taxon distance was available for both *A. fulva* and the 18-chromosome form of *A. 'rudis'*.

Taxa	C.P.	M(22)	M(18)	*fulva*
Coastal Plains	.01±.01	.24±.02	.32±.02	.32±.07
Montane (n = 22)		.03±.01	.19±.03	.12±.04
Montane (n = 18)			(.01)	.42±.12
fulva				(.15)

preparing Table 4 as these would have contributed spuriously to the variance estimates.

Table 4 shows that the various chromosomally-characterised taxa in this study indeed show internal genetic unity while differing significantly from each other. However, the strong differentiation between *fulva* and the 18-chromosome Montane *'rudis'*, which have similar, possibly identical, karyotypes, suggests that allelic differentiation is possible in ants in the absence of chromosomal reproductive isolating mechanisms.

Note that the D_m values in Tables 3 and 4 are not absolute values, because the four loci were far from a random sample. Of some eight systems that stained well, these were the only polymorphic ones in trial colonies and were selected for study.

Discussion

The 18-, 22-, and 20-chromosome 'forms' are reproductively isolated when sympatric, as shown by both the chromosomal and the isozyme evidence, and are thus distinct species despite their close morphological resemblance. There is, of course, a remote possibility that these taxa intergrade in some other portion of their range, but it seems very unlikely that this would be the case. There is a suggestion of limited gene flow between the 20- and 22-chromosome species at Turner's Corner from the *Es* frequencies, and data from the same locus also suggests gene flow between *fulva* and sympatric *'rudis'* populations. However, the chromosomal evidence, as well as that of the *Mdh-a* frequencies, indicates that this gene flow must be extremely restricted, as for sympatric populations of *Drosophila metzii* and *D. pellewae* in Hawaii (Carson et al., 1975b). The sharing of alleles between the three cytotypes of *'rudis'* in Georgia, despite their considerable karyotypic divergence, is reminiscent of the lesser allozymic than chromosomal differentiation between the Hawaiian sibling species *Drosophila setosimentum* and *D. ochrobasis* (Carson et al., 1975), as well as the extremely slight allozymic differentiation between synchronic species of periodical cicads (Krepp & Smith, 1974). The isozymic differentiation among the various 18-chromosome taxa in the apparent absence of karyotypic change is surprising, however.

Evolutionarily, the three Georgian *'rudis'* species would seem to be only distantly related on the basis of both the chromosomal and the allozymic evidence. The narrowness of the Turner's Corner overlap zone suggests that ecological differentiation might be slight, with at least the 20- and 22-chromosome forms occupying the same 'resource peak' (Crozier, 1974b). Gene flow away from the overlap zone could be slowed, enhancing any eliminatory effects of selection, by the altitudinal zonation of neighbouring populations: production of mature sexual brood was increasingly delayed with increasing altitude, suggesting that the mating flights of populations at different elevations would be similarly out of phase.

The observed isozymic differences between the Coastal Plains and both Montane forms is paralleled by similar findings that colour 'forms' actually represent sibling species in the mosquito *Aedes aegypti* (Scott & McClelland, 1975) and the fiddler crab *Uca pugilator* (Selander et al., 1971). However, as noted above, the 18-chromosome species on Black Rock Mountain could not have been detected without chromosomal evidence.

Clusters of sibling species that are extremely similar morphologically seem likely to be common among ants. Thus, allozymic and nest-form differences have been demonstrated between colour 'forms' of the Australian meat ant *Iridomyrmex purpureus* (Halliday, 1975; Greenslade, 1974). Disjunct samples of the Australian *Rhytidoponera metallica* show either a Robertsonian polymorphism with haploid numbers ranging from 17 to 23, or karyotypes with 11-12 pairs of chromosomes (Crozier, 1969b; Imai, Crozier & Taylor, unpublished); examination of the intergrade zone would be interesting in this case. Tunisian *Camponotus compressus* have been reported as having

n = 20 (Hauschteck-Jungen, *in* Crozier, 1975), whereas Indian *C. compressus* are said to have n = 10 (Kumbkarni, 1965). One colony of the European *Myrmica sulcinodis* had uniformly n = 28, another n = 24 (Hauschteck-Jungen, *in* Crozier, 1975). *Conomyrma (=Dorymyrmex) bicolor* (from Arizona) and *C. ?thoracicus* (from Peru) have morphologically indistinguishable workers but divergent karyotypes (n = 13, 9, respectively) (Crozier, 1970). Because the speed of chromosomal evolution varies greatly from one ant genus to the next (Crozier, 1975), karyotypic evidence will certainly not always detect sibling species, but multi-locus studies probably would.

The difficulty that would be found in detecting the presence of three, not one, species of *rudis*-group *Aphaenogaster* from the allozyme data alone suggests that isozyme genotype-environment studies in ants that rely solely on a small number of loci could result in data reflecting not variation within one species but rather geographic differences in relative abundances between species. In the absence of chromosomal data and the study on the Turner's corner overlap zone, the allozyme frequencies involved would certainly indicate marked genotype-environment interactions within one species.

Allozyme variation in other Hymenoptera has been found to be extremely restricted. Little variation was found in ants of the *Formica rufa* (Pamilo et al., 1975) and *Rhytidoponera chalybaea* (P.S. Ward, pers. comm.) groups, and in a variety of solitary bees and wasps (Metcalf et al., 1975), and no variation at all in three bee species (Snyder, 1974). Nine or more loci were examined for each of these studies. However, investigators have found polymorphic loci in other species fairly readily, such as the honey bee *Apis mellifera* (Mestriner & Contel, 1972) and the fire ants *Solenopsis invicta* and *geminata* (A.C.F. Hung, pers. comm.), suggesting that the restriction of variability may not be general within the order. The case of the *Formica* species is interesting, as this genus is karyotypically one of the most uniform among ants (Crozier, 1975) – could there be a correlation between karyotypic and allozymic variability?

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