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INTRAPOPULATION GENETIC SIMILARITY IN *POGONOMYRMEX CALIFORNICUS* (BUCKLEY) (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Two color morphs of *Pogonomyrmex californicus* (Buckley) were studied at twelve enzyme loci by means of gel electrophoresis to determine whether or not the dark phase morph was genetically distinguishable from the typical morph. The results strongly suggest that, at the loci and in the populations studied, the two morphs are members of the same species. These findings provide additional evidence in support of the synonymy of *P. c. estebanius* with *P. californicus*. The suggestion is made that the dark phase morph may have evolved slightly different isozymes to cope with thermal stress.

INTRODUCTION

Pogonomyrmex californicus (Buckley) is a species of harvester ant common throughout most of the southwestern desert states, especially California and Arizona. Identification of the "typical" *P. californicus* worker presents few problems. It is of comparatively small stature (5.5-6.0 mm in length), has an unarmed epinotum, bears coarse cephalic and thoracic rugae, and has a concolorous, ferruginous red body color. With an eastward progression in range, however, there is an infuscation of the gaster in one or all of a series, ranging from lateral spotting to concolorous black or brown (Cole, 1968). Thus the species is polymorphic with respect to color, with one extreme being represented by totally red individuals (typical) and the other by individuals with head, thorax, petiole, postpetiole, and legs red and the gaster entirely black or brown (dark phase). Wheeler (1914) and Cole (1968) noted size differences between the two forms, with typical being somewhat smaller than dark phase. Creighton (1950), however was unable to detect any size differences that could reliably separate the two forms and concluded that "the two forms are most readily separable on the basis of color. . . ."

Colonies of both typical and dark phase occur throughout California, Arizona, New Mexico, and northern Mexico (Cole, 1968). In some nests, both types are found intermingled with a full range of infuscation. Typical colonies are found mainly in the coastal areas of California and Baja California. In the central Mojave Desert, colonies of dark phase ants are found extending to lower elevations (below 1500 m) than typical colonies, while in southern Arizona, typical and dark phase colonies are sympatrically distributed (Cole, 1968).

Initially, workers of the dark phase variant were assigned subspecific state (*P. c. estebanius*), principally on the basis of their bicolored appearance (Wheeler, 1914). This convention was maintained in a number of subsequent studies of the species (Olsen, 1934; Creighton, 1950; Cook, 1953). Creighton (1950) noted, however, that subspecific status for *estebanius* was not without its questionable aspects—and stated that "it would be much more in keeping with the concept of *estebanius* as a geographical race if it showed a more distinctive range of its own." He believed that such a distinctive range might lie in northwestern Mexico. Cole (1968) has synonymized *P. c. estebanius* with *P. californicus* on the premise that the color difference that separates the two is within the "established limits of intraspecific variation." He supports this classification with the following facts: 1) no distinct range for *estebanius* has been found; 2) the two types have been shown to exist within the same nest; and 3) no characters have been found which could provide *estebanius* with the status of a species.

With these unresolved conflicts in the existing data, it was felt that new biochemical genetic evidence could provide further clarification of the taxonomy of this species. We therefore sought to determine those levels of genetic variability between the two morphs that were not an expression of environmental heterogeneity (Johnson et al., 1969). Such findings would provide us with a further test of Cole's statement that *estebanius* lacks the characteristics of a species distinct from *californicus*. Electrophoretic analysis methods were employed to assess quantitatively the level of genetic variability between the two forms.

MATERIALS AND METHODS

Typical and dark phase workers of *Pogonomyrmex californicus* were collected from nests in the central Mojave Desert near Pearblossom, Los Angeles County, California. The collections were restricted to an area of about eight square kilometers to reduce the possibility of clinal gradients being factors in any observed variation (Johnson et al., 1969). Approximately 250 individuals were taken from fifteen nests in the area in April-May 1976.

Twelve structural genes coding for enzymes were studied, as follows: Esterase (ESp & ESr), four loci each; phosphoglucose isomerase (PGI), one locus; a malate dehydrogenase (MDH), one locus; phosphoglucomutase (PGM), two loci. Whole live ants were homogenized in 50 µl of distilled water by means of a small mortar and pestle. Pooled samples for homology tests were obtained by homogenizing together a single individual from each of two populations. The resulting homogenate was absorbed directly into 4 x 7 mm rectangles of Wratten No. 1 filter paper and subjected to horizontal starch gel electrophoresis in a 12.4% gel for 3-3.5 hours at a maximum current of 50 mA. A discontinuous buffer system was employed (Poulik, 1957). Standard procedures for isozyme assay were used (Johnson et al., 1969; Shaw and Prasad, 1970; Koehn, 1967).

RESULTS

The observed genotypes and allelic frequencies were determined by direct counts of the various bands developed in the gels. A conservative scoring procedure was used by which only unquestionably distinct bands were counted. Alleles at each locus were identified with a relative electrophoretic mobility value; the most common, slowest allele at each locus was designated 1.00. Alleles slower than this were designated 0.95; faster alleles were designated 1.05. The estimated gene frequencies given in Table 1 were calculated from the raw data using the method of Hubby and Lewontin (1966).

Two significant results emerge from the data presented in Table 1. First, there are low levels of enzyme polymorphism in all sample studied, with a maximum of three out of twelve loci clearly polymorphic using the 1% criterion of Ayala et al. (1970). Second, each locus examined had an allele in common between samples. This is particularly evident at the MDH and PGI loci, which were also consistently monomorphic. The esterase loci, despite the presence of a few rare alleles, each demonstrated an allele in common between samples. The common alleles were revealed by homology tests. The PGM-2 locus is unique in that the 1.00 allele is not represented in any of the dark phase samples.

Estimates of the genetic similarity of the two populations were calculated using Rogers' formula (1972) for similarity and Hedrick's formula (1971) for probability of genotype identity. Rogers' coefficient assumes values from zero for similar populations to one for populations that are completely dissimilar genetically. Hedrick's formula compares genotype, rather than gene, frequencies. The probability values range from one for identical genotypes to zero for highly dissimilar genotypes. The results of the tests were as follows: Rogers' coefficient: 0.04: Hedrick's probability: 0.98.

TABLE 1. *Estimated allelic frequencies in populations of the color morphs of* Pogonomyrmex californicus *(Buckley).*

		Populations	
Locus	*Allele*	*Typical*	*Dark Phase*
ESp-1	0.95	0.09	—
	1.00	0.90	1.00
	1.05	0.01	—
ESp-2	1.00	1.00	1.00
ESp-3	1.00	1.00	1.00
ESp-4	1.00	0.99	1.00
	1.05	0.01	—
ESr-1	0.95	0.09	—
	1.00	0.91	1.00
ESr-2	1.00	1.00	1.00
ESr-3	1.00	1.00	1.00
ESr-4	1.00	0.98	1.00
	1.05	0.02	—
MDH	1.00	1.00	1.00
PGI	1.00	1.00	1.00
PGM-1	1.00	1.00	1.00
PGM-2	1.00	0.66	—
	1.05	0.34	1.00

DISCUSSION

The principal object of this investigation was to ascertain the level of genetic variability between the two morphs, exclusive of those variations resulting from environmental heterogeneity. The electrophoretic data and the results of their analysis strongly suggest that the observed value of similarity was in no case significantly different from the value expected for members of the same species. While speciation does not seem to require a large genetic change, the amount of change detected here does not indicate any divergence between the two morphs. The slight variations noted at loci other than PGM-2 may be the result of experimental error, band scoring discrepancies, or restricted sample size.

Polymorphism at the esterase loci is to be expected. Such enzymes, which utilize substrates originating in the environment, are more variable than those utilizing specific metabolically produced substrates (Johnson, 1973). The total lack of polymorphism in the dark phase samples could be the result of the conservative scoring procedure and the deliberate attempt to reduce clinal variation. The average of observed levels of polymorphism in all samples, 12.5%, suggests that outbreeding and gene flow are quite restricted in these populations and that selection for certain specialized genotypes may have taken place.

It is interesting to note that the two forms differ most at the PGM-2 locus. Such a difference does not represent potential speciation; however, it could indicate that the two morphs have evolved slightly different physiological mechanisms to cope with environmental heterogeneity. However, the limited data does not, as yet, enable us to predict whether this particular polymorphism is a common enough occurrence to be significant in the ecology of *P. californicus*. Further work will be required to determine, for example, if there is a correlation between this variation and the "thermophilous" character of the dark phase morph (Creighton, 1950).

While electrophoresis currently represents the best approach to the study of genetic variation, the method is not without its sources of error. Principal among these is that some amino acid substitutions may occur that do not have any detectable effect on a protein's net charge, thus leading to an underestimate of a sample's variability by a factor of three (Lewontin, 1973). Another source of error that cannot be corrected for easily is that loci which demonstrate significant variability may not have been assayed; assay techniques are available for only a limited number of enzymes. This problem is compounded by the fact that color polymorphism in insects is frequently controlled by a single gene, the isozymes of which (if any) may not detectable. Because of these and other problems, we cannot ignore the possibility that there is a genetic difference between the two morphs; significant variation may have been obscured or undetected. It is primarily for these reasons that the result of the present study may be considered highly suggestive rather than conclusive.

On the basis of the biochemical genetic evidence alone, we could not preclude considering *estebanius* as a subspecies of *P. californicus* since the two are almost certainly conspecific. However, these results do support Cole's statement that *estebanius* does not display any characters which distinguish it as a species distinct from *P. californicus*. Thus these findings, in combination with those of Creighton (1950) and Cole (1968), provide additional justification for the synonymy of *P. c. estebanius* with *P. californicus*.

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