COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
J. Aust. ent. Soc., 1981, **20:** 177-183

ALLOZYME VARIATION, COLONY STRUCTURE AND GENETIC RELATEDNESS IN THE PRIMITIVE ANT *NOTHOMYRMECIA MACROPS* CLARK (HYMENOPTERA: FORMICIDAE)

PHILIP S. WARD* and ROBERT W. TAYLOR

Zoology Department, University of Sydney, N.S.W. 2006.
Division of Entomology, CSIRO, P.O. Box 1700, Canberra City, A.C.T. 2601.

Abstract

A survey of electrophoretically detectable genetic variation in the only known population of *Nothomyrmecia macrops* Clark (from N.W. Eyre Peninsula, South Australia) revealed one polymorphic locus (amylase) among 16 studied, and a mean heterozygosity ($\bar{H}$) of 0.032, a value lower than those reported for most other insects, but consistent with previous comparable estimates for ants and other Hymenoptera. Variability at the amylase locus is considerable, with 4 alleles present at frequencies > 0.05. There is no evidence of inbreeding, genotype frequencies being in Hardy-Weinberg equilibrium. An examination of genotype frequencies within 4 nests demonstrates that workers from the same nest are not always sibs, and a regression analysis of relatedness among worker nestmates yields a mean estimated coefficient of relatedness (b) of 0.251, based on 2 different diallelic combinations. The same analysis applied to samples of workers taken foraging on separate, single *Eucalyptus* trees gives a mean b of only 0.084 (based on three diallelic combinations), a result consistent with field observations of an overlap of foraging areas (and probably low territoriality) between nests. The relatedness estimates must be interpreted with caution because of limited sample sizes. Additional field studies and electrophoretic analyses are required to assess the possible contributions to the observed patterns of relatedness from polygyny, multiple insemination of the queen(s), and worker interchange between colonies.

Introduction

The origin and early stages in the evolution of formicid sociality remain rather obscure and somewhat intractable, partly because of the consistently eusocial nature of contemporary species (apart from a few social parasites). Attempts to analyse possible directions of social evolution by comparative studies are likely to be less satisfactory for ants than for wasps and bees, where wide variation in degree of sociality can be found within families or even genera (Wilson 1971; Michener 1974). In this regard, the recent rediscovery of the "living-fossil" ant *Nothomyrmecia macrops* Clark (Taylor 1978) is of particular interest, not only because of the peculiar, mostly primitive morphology of this species, but also because its behaviour might include components representing early stages in formicid social evolution.

It is now known that *N. macrops* is fully eusocial, with a moderately differentiated queen caste (Taylor 1978). However, many aspects of its social behaviour remain unstudied, including those relevant to recent theory concerning the evolution of eusociality in the Hymenoptera. Kin-selection theory stresses the peculiar asymmetries of relatedness associated with haplodiploidy, which are thought to encourage female altruism (Hamilton 1964, 1972; Trivers and Hare 1976). Parental manipulation of offspring (Alexander 1974) has been cited as a competing model, which might work in concert with kin-selection (e.g. Craig 1979). From the point of view of relatedness, the origin and maintenance of social behaviour is theoretically favoured in outbred populations with monogynous colonies containing a queen who has mated only once. Thus, it is of some interest to examine population and colony structure in putatively primitive species like *N. macrops*, to investigate whether these and other conditions theoretically associated with the early evolution of eusociality are retained in morphologically pleisiomorphic species.

This report considers genetic variability, colony structure, and relatedness in the only known extant population of *Nothomyrmecia*, and is based on electrophoretic analysis of gene-enzyme variation, supplemented by field studies.

*Present address: *Department of Entomology, The University of California, Davis, California 95616, U.S.A.*

Collection methods

Nothomyrmecia specimens were collected from mallee woodland in the north west of Eyre Peninsula, South Australia, in November 1977. The collection site is described elsewhere (Taylor 1978). All colonies sampled and otherwise left undisturbed, and those fully excavated, were mapped, as were the locations of tree trunks from which nocturnal foragers were gathered (Fig. 1). Only a limited number of individuals were available for electrophoresis. These included workers and a few queens, but no males.

The following material was examined electrophoretically:

(1) Forty-five workers sampled from 4 different nest series numbered 2882, 77.715, 77.716 and 77.717, plus a dealate queen from series 77.717*.

(2) Nine series of workers, including 108 individuals in all, collected foraging at night from 9 different *Eucalyptus oleosa* trees (Fig. 1). Members of each series are referred to below as "co-foragers".

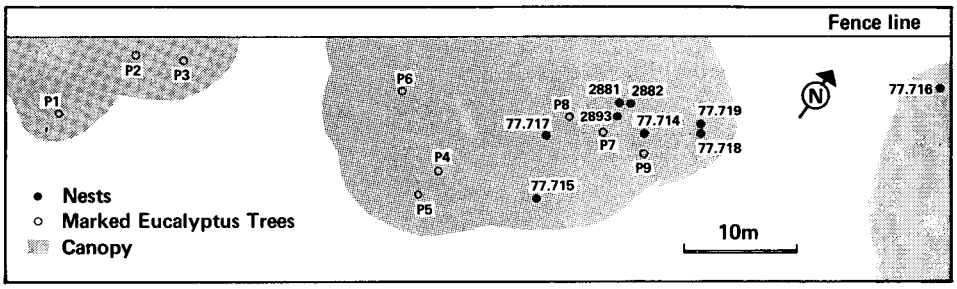
Electrophoresis

Specimens for analysis were stored frozen at −25° C. Whole individuals were ground in distilled water for electrophoresis. The crude homogenate was absorbed onto circles of Whatman No. 1 filter paper, and run on 7% polyacrylamide "disc" (tube) gels (Davis 1964; Ornstein 1964) with a bisacrylamide/acrylamide ratio of 1:30. A single ant contained sufficient protein for several enzyme assays. The electrode buffer was 0.005 M Tris-Glycine (pH 8.3) and the final gel buffer concentrations were either 0.02 M Tris-Borate or 0.375 M Tris-HCl (both pH 8.9) (Ward 1980). Amylase was scored on 6% gels, using the Tris-Borate gel buffer, to which 1 mM EDTA was added. The 14 enzyme systems listed below were examined using standard histochemical staining techniques (Shaw and Prasad 1970; Harris and Hopkinson 1976). Bromphenol blue was used as a mobility reference with all systems.

Results

Allozyme variation and levels of variability

Thirteen enzyme systems, representing 15 putative loci [(1) superoxide dismutase; (2) malate dehydrogenase; (3) glucose-6-phosphate dehydrogenase; (4) hexokinase; (5) xanthine dehydrogenase; (6) aldehyde oxidase; (7) α-glycerophosphate dehydrogenase; (8, 9) lactate dehydrogenase-1, lactate dehydrogenase-2; (10) malic enzyme;



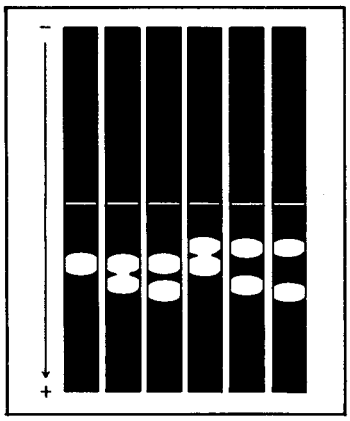


FIG. 1 (above)—*Nothomyrmecia macrops:* Map of collection site showing locations of nest entrances and of *Eucalyptus* trees from which foragers were collected. Nests with ANIC numbers (prefixed 77.) were fully excavated for behavioural research and sampled for this study; those numbered otherwise were incompletely excavated or were sampled by collection of departing foragers. Dotted lines indicate approximate boundaries of dense *Eucalyptus oleosa* canopy. Other unstudied and unmapped nests were present.

FIG. 2 (left)—*Nothomyrmecia macrops:* Diagrammatic representation of typical amylase isozyme variants; putative genotypes are (from left to right): MM, HM, FM, MS, HS and FS. The heavily staining amylase variants are anodal to a much fainter monomorphic band.

*Accession numbers prefixed 77. are those of the Australian National Insect Collection (ANIC) ant section. Number 2882 is Ward's accession number. Other numbers in Ward's records relate to ANIC numbers as follows: ANIC 77.715 = Ward 2886, 77.716 = 2887, 77.717 = 2888. All records of other observations and research on ants from these colonies have been or will be recorded, and reported where applicable, using ANIC numbers.

(11) phosphoglucomutase; (12) glutamate oxaloacetate transaminase; (13) leucine aminopeptidase; and (14, 15) esterase-1 and esterase-2] showed no variation within samples, which varied in size from 8 to 108 workers per locus. The mean number of worker genomes sampled per locus for these 15 loci was 116.9. A further system, amylase (AMY), exhibited considerable isozyme variation (Fig. 2), in a pattern which is strongly suggestive of codominant alleles following Mendelian inheritance. Four alleles were detected, designated S,M,H, and F, in order of increasing mobility. Individuals ~~genomes~~ displayed either 1 (homozygote) or 2 (heterozygote) bands. Frequencies of postulated genotypes are in close accord with Hardy-Weinberg expectations (Table 1). The average heterozygosity at this locus is 0.519, calculated from allele frequencies of 0.134, 0.663, 0.141, and 0.062.

These results indicate that this population of *Nothomyrmecia* is polymorphic at 1/16 or 6.25% of all loci examined. Mean heterozygosity ($\bar{H}$) (Nei and Roychoudhury 1974), averaged over all 16 loci, is 0.032 $\pm$ 0.032 S.E. This is very close to the observed frequency of heterozygotes (0.034). Wright's (1922) inbreeding coefficient (*F*) calculated for the AMY locus, yields a value of -0.058, which is not significantly different from zero ($\chi^2_6 = 1.54$, $P > 0.5$; see Li and Horvitz 1953).

Genetic relatedness within nests and among co-foragers

The available data on genotype frequencies in single-nest series are given in Table 2. These suggest that not all colonies consist simply of a single, once-inseminated queen and her offspring. One colony (No. 2882) evidenced more than 2 genotypes, and in 1 of the remaining 3 colonies (77.717) genotypic proportions differed significantly from 0.5 ($\chi^2 = 5.33$, $p \lesssim 0.05$). In addition, the queen's genotype (MM) in colony 77.717, when compared with those of the workers (MM and MS), is not consistent with her having mated only once (assuming that the colony had received no genetic input from other undetected queens or mated workers and their male consorts; and that workers immigrant from other colonies, and thus of separate parentage, were not present—these matters are discussed in detail below). This queen was dissected and the presence of well-developed ovaries, corpora lutea, and sperm in the spermatheca, confirmed her status as a functional gyne.

TABLE 1

GENOTYPE AND GENE FREQUENCIES AT THE AMYLASE (AMY) LOCUS IN *NOTHOMYRMECIA MACROPS*. EXPECTED GENOTYPE FREQUENCIES ARE CORRECTED FOR SMALL SAMPLE SIZE USING LEVENE'S (1949) FORMULA.

Genotype	Genotype frequencies Observed	Expected		
SS	0	2.69	5.24	
FS	3	2.55		$\chi^2 = 4.72$
MS	28	27.27		df = 3
HS	10	5.80		n.s.
MM	69	67.14		
HM	27	28.70		
FM	10	12.62		Gene frequencies
HH	0	2.98	6.22	f(S) = 0.134
FH	6	2.68		f(M) = 0.663
FF	0	0.56		f(H) = 0.141
	153			f(F) = 0.062

Comparative regression analyses of relatedness within nests and within co-foraging worker series were executed. Since the relatedness (*b*) of individual(s) Y to individual X can be thought of as the regression of Y's additive genotype on that of X (Hamilton 1972), codominant electrophoretic alleles are potentially useful for assaying kinship (Orlove 1975; Craig and Crozier 1979; Pamilo and Varvio-Aho 1979). Following Pamilo and Varvio-Aho (1979) and Pamilo and Crozier (in prep.), Y is the mean additive genotypic value of all potential interactants (such as members of a colony or of a group of foragers) except that of the X under consideration. The data points are weighted so that the total number of observations corresponds to the

number of groups. All data must be in diallelic form; hence several combinations of the AMY alleles were used, in which the frequency of the most common allele class was less than, or equal to, 0.80 in the groups under analysis. These combinations were:

Combination	Alleles			Groups analysed
1	M	vs	S,H,F	Co-foragers, nestmates
2	M,F	vs	S,H	Co-foragers
3	M,S	vs	H,F	Co-foragers
4	M,H	vs	S,F	Nestmates

The raw genotype data for 4 groups of nestmates and 9 groups of co-foragers are given in Tables 2 and 3, respectively.

Table 2
AMY GENOTYPE FREQUENCIES IN FOUR COLONIES OF *NOTHOMYRMECIA MACROPS*

Colony	Sample size	Genotype		
		MM	MS	HM
2882	8 workers	6	1	1
77.715	12 workers	7	5	—
77.716	13 workers	10	3	—
77.717	12 workers	2	10	—
77.717	1 dealate queen	1	—	—

The results of the regression analyses are given in Table 4. The mean b for intranidal workers, averaged over 2 diallelic combinations, is 0.251 (mean S.E. 0.262). For co-foragers it is 0.084, with a mean S.E. of 0.165 (3 diallelic combinations). These figures must be interpreted with caution because of non-independence of the b estimates within each sample. None of the b values is significantly different from zero. However, the S.E. estimate used here is a rather conservative one, particularly for low values of b (Pamilo and Crozier in prep.). The results do indicate that nestmates are not always full sibs (i.e. $b \neq 0.75$). Moreover, the lower mean value of b for co-foragers is compatible with field observations that workers foraging on a single tree do not always originate from the same nest.

Discussion

Although *N. macrops* appears genetically less variable than most insects previously studied using standard electrophoretic techniques (see e.g. reviews by Powell 1975; Nevo 1978), the estimated heterozygosity ($\hat{H} = 0.032$) is well within the range of genetic variation reported for other Hymenoptera. The mean heterozygosity for 53 species covered by such studies and reviewed in Ward (1980) is 0.036 ± 0.004 S.E. For 21 ant species the mean $\hat{H}$ is 0.040 ± 0.005 S.E. (data from Pamilo *et al.* 1978; Halliday 1978; Ward (1980). Thus, despite apparently being a "living-fossil", possibly the sole survivor of an ancient lineage (Taylor 1978), *N. macrops* shows no evidence of being genetically depauperate relative to other ants. Considerable genetic variability is also present in populations of another "living-fossil" arthropod, the xiphosurid *Limulus polyphemus* L. (Selander *et al.* 1970).

There is considerable variability at the AMY locus in *N. macrops*, the effective number of alleles, n_e (Crow and Kimura 1970) being 2.08. If the observed variation is selectively neutral, then calculations based on the relationship between n_e and effective population size, N_e ($n_e = 4 N_e \mu + 1$; see Crow and Kimura 1970, p. 324), suggest an N_e in the order of 10^4 (assuming that μ, the mutation rate, approximates 10^{-5}).

TABLE 3
AMY GENOTYPES AMONG *NOTHOMYRMECIA* CO-FORAGERS. EACH SERIES REPRESENTS WORKERS COLLECTED ON A SINGLE *EUCALYPTUS OLEOSA* TREE

Series	No. workers	Genotype						
		MM	MS	HM	FM	HS	FS	FH
P1 (2864)	12	6	1	3	—	2	—	—
P2 (2865)	12	7	1	2	2	—	—	—
P3 (2866)	12	8	—	3	—	1	—	—
P4 (2868)	12	5	—	4	2	1	—	—
P5 (2869)	6	4	1	1	—	—	—	—
P6 (2870)	13	—	1	7	—	—	—	5
P7 (2871)	11	4	3	2	2	—	—	—
P8 (2872)	6	3	1	1	—	1	—	—
P9 (2873)	24	7	1	3	4	5	3	1

On the other hand, field observations suggest that our *N. macrops* population is small and localised. We believe it could, as known, consist of no more than about 100 colonies. Either the effective population size is much larger than this, or some form of balancing selection is acting at the AMY or a closely-linked locus.

Several factors could be responsible for the apparently low level of genetic relatedness among workers from the same nest. These include (1) polygyny, (2) multiple insemination of the queen(s), and (3) worker interchange between colonies. In the following discussion we consider these possibilities in turn.

(1) Our field collections suggest that *N. macrops* colonies are generally monogynous. Five mature colonies were fully excavated: 4 of these contained a single, apparently functional, dealate queen. A queen was not recovered from the fifth nest, but was probably overlooked during excavation. A sixth colony excavated in October 1978 by Taylor, in company with C. P. and E. F. Haskins, also contained a single queen. In addition we collected 2 incipient colonies; 1 consisted of a single queen, the other contained 2 co-operating queens. Thus the available facts, while scanty, indicate monogyny in mature colonies, although colony foundation can involve pleometrosis.

TABLE 4
REGRESSION ESTIMATES OF RELATEDNESS (b) AMONG CO-FORAGING WORKERS AND WORKER NESTMATES OF *NOTHOMYRMECIA MACROPS*, BASED ON AMY GENOTYPE DATA

Group	Allele combination	b	S.E.	
Nestmates	no. 1	.162	.333	
	no. 4	.340	.190	$\bar{b}$ = .251
Co-foragers	no. 1	.123	.171	
	no. 2	—.097	.134	$\bar{b}$ = .084
	no. 3	.225	.191	

Incidentally, most reported instances of primary pleometrosis in ants involve species whose queens are initially fully-winged, and undergo aerial dispersal. It is usually assumed that associating dealated colony-founding queens are unrelated. *N. macrops* queens are brachypterous (Taylor 1978) and it is possible that limited vagility results in colony-founding associations between relatives. Chance alone would contribute towards this.

If functional monogyny follows primary pleometrosis, then a residue of the ousted supernumerary queen's offspring will maintain a mean relatedness between workers lower than 0.75. However, as the monogynous colony enters a log phase of

growth, the proportion of nonsiblings should drop fairly rapidly, and relatedness should rise well above the empirical estimates obtained in this study. Also, the supernumerary workers could reasonably be expected to die long before the colony itself expired, in which case they would make no contribution at all to analyses based on workers from old colonies.

There is a distant possibility that *N. macrops* nests are polygynous as a result of reproduction by mated workers. We consider this rather unlikely. However, it would be difficult to detect without exhaustive dissections of all workers from 1 or more colonies. No workers from excavated nests were examined by us for ovarian development; but a single foraging worker was dissected and found to have rather well-developed ovaries, with 7 ovarioles (9 to 10 ovarioles were found in 5 queens which were dissected). The outstretched ovaries were 0.6 mm long, with about 5 conspicuous (i.e. opaque) oocytes, but no corpora lutea. A spermatheca was present but empty. The absence of corpora lutea suggests that this individual, as dissected, could have produced only trophic eggs (as a forager it is unlikely to have been reproductive in any case). Nevertheless it is clear that some workers at least possess all the major organs required for reproduction. The brachypterous nature of the queens suggests that mating probably occurs near the mother nest. If so, workers abroad from their nests might be in close proximity to sexually active males during periods of reproductive activity, and thus perhaps liable to insemination. Taylor (1978) has observed the occasional production by workers of both trophic and "apparently reproductive" eggs in observation nests. Reproductive eggs in this context might normally be genetically haploid and thus destined to produce male adults.

(2) It is difficult to evaluate the possibility that multiple insemination of a single functional queen is responsible for the observed patterns of within-nest relatedness. Detailed studies of mating behaviour and/or allozyme analyses of unequivocally unimaternal offspring, are necessary. Information on this important aspect of ant reproductive biology is very scant.

(3) Worker interchange could well be responsible for increasing genotypic diversity in colonies. As corroborative evidence we cite the following observations.

(i) A lack of inter-colony aggression has been observed in the laboratory, where workers from several different colonies lived amicably in a single observation nest chamber (Taylor 1978).

(ii) There is a sharing of foraging areas in the field. Field observations of workers homeward-bound at dawn revealed that co-foragers can come from more than one nest.

(iii) A nearest-neighbour analysis of distances between nest entrances in an area approximately 10 × 20 m in the north eastern half of the main *Eucalyptus oleosa* stand (see Fig. 1) reveals a dispersion index, R, of 0.888 which, although suggesting slight aggregation, is not significantly different from random (using the significance test of Clark and Evans 1954). One would expect over-dispersion of nests if inter-colony territoriality was important (cf. Brian 1956; Waloff and Blackith 1962; Yasuno 1964).

The evident lack of inter-colony aggression and of territoriality could imply that colonies are polydomous, with each nest site merely a repository for one queen and a portion of the total worker force. Polygyny and unicoloniality are associated with ant species occupying patchily distributed habitats (Hölldobler and Wilson 1977). There is as yet no evidence for this type of colony organisation in *N. macrops*.

There are clearly many aspects of colony structure in *Nothomyrmecia* whose investigation will require more detailed field studies. The present report is preliminary, and necessarily based on limited sample sizes. If *Nothomyrmecia* proves to be more common and widespread, the additional analyses of social structure based on allozyme data will be possible.

The finding that colonies are not simple family units, consisting entirely of full sibs, demonstrates that sociality, even in such an apparently primitive ant as *N. macrops*, is not necessarily contingent upon a matrifilial colony structure involving a once-mated queen.

Acknowledgments

We are grateful to Drs R. H. Crozier and P. Pamilo for comments. Financial support to P.S.W. was provided by a Commonwealth Scholarship and by the University of Sydney.

References

ALEXANDER, R. D. (1974).—The evolution of social behaviour. *A. Rev. Ecol. Syst.* **5:** 325-383.
BRIAN, M. V. (1956).—Segregation of species of the ant genus *Myrmica*. *J. anim. Ecol.* **25:** 319-337.
CRAIG, R. (1979).—Parental manipulation, kin selection, and the evolution of altruism. *Evolution, Lancaster, Pa.* **33:** 319-334.
CRAIG, R. and CROZIER, R. H. (1979).—Relatedness in the polygynous ant *Myrmecia pilosula*. *Evolution, Lancaster, Pa.* **33:** 335-341.
CLARK, P. J. and EVANS, F. C. (1954).—Distance to nearest neighbour as a measure of spatial relationships in populations. *Ecology* **35:** 445-453.
CROW, J. F. and KIMURA, M. (1970).—*An introduction to population genetics theory*. Harper and Row: New York.
DAVIS, B. J. (1964).—Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121:** 404-427.
HARRIS, H. and HOPKINSON, D. A. (1976).—*Handbook of enzyme electrophoresis in human genetics*. American Elsevier: New York.
HALLIDAY, R. B. (1978).—Genetic studies of meat ants. Ph.D. thesis, University of Adelaide: Adelaide.
HAMILTON, W. D. (1964).—The genetical evolution of social behaviour. *J. theor. Biol.* **7:** 1-52.
HAMILTON, W. D. (1972).—Altruism and related phenomena, mainly in social insects. *A. Rev. Ecol. Syst.* **3:** 193-232.
HÖLLDOBLER, B. and WILSON, E. O. (1977).—The number of queens: an important trait in ant evolution. *Naturwissenschaften* **64:** 8-15.
LEVENE, H. (1949).—On a matching problem arising in genetics. *Ann. Math. Stat.* **20:** 91-94.
LI, C. C. and HORVITZ, D. G. (1953).—Some methods of estimating the inbreeding coefficient. *Am. J. hum. Genet.* **5:** 107-117.
MICHENER, C. D. (1974).—*The social behaviour of the bees*. Harvard University Press: Cambridge, Mass.
NEI, M. and ROYCHOUDHURY, A. K. (1974).—Sampling variances of heterozygosity and genetic distance. *Genetics, Princeton* **76:** 379-390.
NEVO, E. (1978).—Genetic variation in natural populations: patterns and theory. *Theor. Pop. Biol.* **13:** 121-177.
ORLOVE, M. J. (1975).—A model of kin selection not invoking coefficients of relationship. *J. theor. Biol.* **49:** 289-310.
ORNSTEIN, L. (1964).—Disc electrophoresis. I. Background and theory. *Ann. N. Y. Acad. Sci.* **121:** 321-349.
PAMILO, P., ROSENGREN, R., VEPSÄLÄINEN, K., VARVIO-AHO, S. and PISARSKI, B. (1978).—Population genetics of *Formica* ants. I. Patterns of enzyme gene variation. *Hereditas* **89:** 233-248.
PAMILO, P. and VARVIO-AHO, S. (1979).—Genetic structure of nests in the ant *Formica sanguinea*. *Behav. Ecol. Sociobiol.* **6:** 91-98.
POWELL, J. R. (1975).—Protein variation in natural populations of animals. *In* Dobzhansky, T., Hecht, M. K. and Steere, W. C. (Eds). *Evolutionary biology* **8:** 79-119. Plenum Press: New York.
SELANDER, R. K., YANG, S. Y., LEWONTIN, R. C. and JOHNSON, W. E. (1970).—Genetic variation in the horseshoe crab (*Limulus polyphemus*), a phylogenetic "relic." *Evolution, Lancaster, Pa.* **24:** 402-414.
SHAW, C. R. and PRASAD, R. (1970).—Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochem. Genetics* **4:** 297-320.
TAYLOR, R. W. (1978).—*Nothomyrmecia macrops:* a living-fossil ant rediscovered. *Science N.Y.* **201:** 979-985.
TRIVERS, R. L. and HARE, H. (1976).—Haplodiploidy and the evolution of the social insects. *Science N.Y.* **191:** 249-263.
WALOFF, N. and BLACKITH, R. E. (1962).—The growth and distribution of the mounds of *Lasius flavus* (Fabricius) (Hym: Formicidae) in Silwood Park, Berkshire. *J. anim. Ecol.* **31:** 421-437.
WARD, P. S. (1980).—Genetic variation and population differentiation in the *Rhytidoponera impressa* group, a species complex of ponerine ants (Hymenoptera: Formicidae). *Evolution, Lancaster, Pa.* **34:** 1060-1076.
WILSON, E. O. (1971).—*The insect societies*. Harvard University Press: Cambridge, Mass.
WRIGHT, S. (1922).—Coefficients of inbreeding and relationship. *Amer. Nat.* **56:** 330-338.
YASUNO, M. (1964).—The study of the ant population in the grassland at Mt. Hakkôda. II. The distribution pattern of ant nests at the Kayano grassland. *Science Reports Tohôku Univ., Sendai, ser. 4 (Biol.)* **30:** 43-55.

[*Manuscript received* 15 *April* 1980. *Revised* 9 *February* 1981.]