Reproductive Strategies in Ants: A Comparison of Single-Queened Versus Multiple-Queened Species in the Subfamily Dolichoderinae (Hymenoptera: Formicidae)

By

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ABSTRACT

Among ants, social structure varies considerably within the confines of eusociality. One important trait that varies is the number of queens per colony. Most species are single-queened (monogynous), but a sizeable minority are multiple-queened (polygynous). This study compares monogynous and polygynous species within one subfamily of ants, the Dolichoderinae. Three important questions were addressed. First, how does polygyny bear upon the genetical theory of the evolution of eusociality? Second, what are the ecological advantages of polygyny? Third, how is intraspecific genetic variation related to social structure in ants, specifically, and in Hymenoptera in general?

Three species were studied intensively: Conomyrma insana, Conomyrma bicolor, and Iridomyrmex pruinosum. Starch gel electrophoresis was performed on individual ants of each species from a number of localities. Frequency distributions of the genotypes present within colonies were compared with those predicted by four alternative models, to determine gyny probabilistically for each species. Conomyrma insana is monogynous. Conomyrma bicolor and Iridomyrmex pruinosum are frequently polygynous. These conclusions were corroborated by field observations and laboratory rearing studies.

Population genetic structure was analyzed for animals collected at 6 sites in the Palomar Valley, San Diego County, California. The prediction that polygynous species have relatively viscous population structures was tested. This very important prediction is based on W. D. Hamilton's haplodiploidy/kin selection model of the evolution of eusociality. If the prediction fails to be upheld, then the relatively common occurrence of polygyny is a major argument against Hamilton's genetical theory.

Analyses of electrophoretic data using F-statistics and modified genetic distance (D) measures showed that one of the polygynous species, I. pruinosum, has a viscous population structure, as predicted, whereas the second polygynous species, C. bicolor, does not. A trait group selection model of the evolution of eusociality is presented. While it is more general than the kin selection model, its predictions concerning polygyny are identical with those under kin selection.

Field studies of ant activity following chaparral fire were conducted to determine whether polygyny confers a colonizing advantage on species. Ant activity at baits was censused periodically for fifty-three months following a small chaparral fire near Hot Springs Mountain, San Diego County, California. Polygyny is only one of many traits that must be considered when predicting ant activity following chaparral fire. Other important characteristics include tolerance of environmental extremes, nesting habits, and vulnerability to predation.

Laboratory rearing studies were conducted to determine whether polygyny confers growth or survival advantages during the early stages

of colony founding. Colonies of Conomyrma bicolor were established with varying numbers of newly mated queens and workers. It was found that C. bicolor females were more likely to lay eggs when accompanied by other queens and (or) workers. Unfed queens did not survive long enough to produce workers, suggesting that colony founding by unaided queens is unlikely. C. insana queens did not tolerate polygyny and appeared capable of founding independently.

Intraspecific population genetic variability in the three species studied was low, compared with non-hymenopteran insects, and similar to that among other Hymenoptera. Polygynous ant species appear no more variable than monogynous species, either among the species in this study or among those reported in the literature.

The species in this study, along with other Hymenoptera reported in the literature, were grouped by social structure as either solitary, primitively eusocial, or eusocial. The prediction that low levels of genetic variability are a consequence of eusociality was tested. Solitary and advanced eusocial species did not differ. However, primitively eusocial species did have significantly lower variability than either of the other two groups. Low levels of variability may be associated with the early stages of the evolution of eusociality.

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The ideas were first born in response to a straw man unwittingly constructed by Roy Rappaport of the Department of Anthropology at the University of Michigan, long before I began my doctoral work at Berkeley. They were nurtured through the formative stages by Dick Alexander, also of the University of Michigan.

My ideas bloomed at Berkeley with the help of many faculty and graduate students, particularly of the ecology lunch group. I have especially benefited from classes and discussions with Howell Daly, Bill Lidicker, George Oster, and Bonnie Bowen. Among other things, Dr. Daly has kept me honest about Hymenoptera facts, Dr. Lidicker introduced me to much of the literature on social altruism and genetic ecology, and Dr. Oster demystified mathematical modelling for me. Bonnie is only one of many fellow students with whom I have exchanged ideas. However, she and I have had remarkably parallel experiences and ideas, although working on very different systems.

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CHAPTER 1. Introduction

All ants and termites, as well as some bees and wasps, are "eusocial". By definition of the term, their colonies include at least two female castes: one which is more fertile (the queen caste) and one which is less fertile or sterile (the worker caste). Many eusocial species, particularly among ants, are characterized by having only one queen per colony (monogyny), while others have multiple colony queens (polygyny). Polygynous species are most common among wasps, but comprise a sizeable minority among ants.

This dissertation addresses three major questions about ant social structure. First, how does polygyny bear upon the genetical theory of the evolution of eusociality? Second, what are some of the ecological advantages of polygyny? And third, how is the level of intraspecific genetic variation related to social structure in ants, specifically, and in Hymenoptera in general?

Monogyny and polygyny are terms which refer simply to the number of queens per colony, but give little information about colony founding and subsequent development. It is useful to discuss a variety of other terms relating to colony founding and development here, as these will be used throughout this dissertation. Colonies may be founded by single queens (haplometrosis) and remain monogynous. Such monogyny is considered primary. Alternatively, colonies may be founded by groups of queens (pleometrosis) and later reduced to a single-queened condition; these are considered to exhibit secondary monogyny. Symmetrically, colonies founded by groups of females that retain their multiple queens show primary polygyny, while colonies founded by single queens may subsequently incorporate additional queens and exhibit

secondary polygyny. Finally, queens may form colonies independently or or, when accompanied by workers, by swarming or budding. In the case of independent, haplometrotic, colony founding, females may rear the first brood withour foraging. Such colony founding is called claustral and is the dominant means of colony founding in ants.

Monogynous, claustral colony founding has provided the paradigm on which recent genetic theories of the evolution of eusociality in the Hymenoptera are based (see especially Hamilton, 1964). However, polygyny is both common (particularly among wasps) and evolutionarily widespread, (particularly among ants). Polygyny has been noted to occur in species of at least five of the seven major subfamilies of the family Formicidae (to which all ants belong). Within each subfamily, it occurs among many genera, often occurring in one of two sibling species but not the other (Hölldobler and Wilson, 1977). In those cases where species of one of the most primitive and wasp-like subfamilies (Ponerinae) are polygynous, it may be an evolutionarily primitive trait. However, polygyny among the more advanced subfamilies (Pseudomyrmicinae, Formicinae, Dolichoderinae, and Myrmicinae) cannot be considered primitive and must be polyphyletic.

The polyphyletic nature of polygyny in ants brings up a major question. If monogynous, claustral conditions are essential for Hamilton's genetical theory of the evolution of eusociality, how can we account for the repeated evolution of polygynous species? Hamilton (1972) has recognized the problem which polygyny poses and has suggested that, to be consistent with his genetical theory, polygynous species must have genetically viscous population structures. This

study was designed to test this prediction and, furthermore, to investigate ecological advantages of polygyny which may have favored its repeated evolution.

I have chosen to study several ant species within the small, relatively homogeneous subfamily Dolichoderinae in order to begin to answer the important questions posed by polygyny. Within the subfamily Dolichoderinae, polygyny is common, although not universal. Some species, including the Argentine ant, Iridomyrmex humilis, exhibit extreme polygyny, with hundreds of queens per colony.

To test Hamilton's prediction concerning the population genetic structure of polygynous species, I have studied three species in two genera intensively. Electrophoretically determined allozyme frequencies were analyzed (1) to establish whether colonies of each species were functionally monogynous or polygynous (Chapter 3) and (2) to determine the population genetic structures of these species and analyze their bearing on the genetical model of the evolution of eusociality (Chapter 4).

In order to investigate some of the ecological advantages of polygyny, I conducted a laboratory study on colony founding (Chapter 5) and a field study on colonization and activity of ants following chaparral fire (Chapter 6). The effect of polygyny on colony founding and early growth under laboratory rearing conditions was tested in one of the three species that were found to be polygynous in studies on genetic structure. All three of the species whose genetic structure was analyzed were studied in the field along with other common dolichoderines, formicines, and myrmicines to analyze the relationship,

if any, between the number of queens per colony and colonizing ability following chaparral fire.

Finally genetic data gathered for population structure determination were also analyzed for overall level of variability. This allowed me to answer a third major category of questions posed during the course of the study, dealing with the bases of intraspecific genetic variability among insects (Chapter 2). Hymenoptera are of particular interest because of their haplodiploid sex determination scheme and the variation they exhibit in social structure. To answer questions about the relationship between haplodiploidy, social structure, and genetic variability, the level of genetic variability in the three species studied was compared with existing data on a variety of insect taxa.

LITERATURE CITED

Hamilton, W. D., 1964. The genetical evolution of social behaviour. I and II. J. Theor. Biol. 7:1-52.

Hamilton, W. D., 1972. Altruism and related phenomena, mainly in social insects. Ann. Rev. Ecol. Syst. 3:193-232.

Hölldobler, B. and E. O. Wilson, 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8-15.

CHAPTER 2. Intraspecific genetic variation and haplodiploidy, eusociality, and polygyny in the Hymenoptera

INTRODUCTION

A variety of theories, both neutralist and selectionist, have predicted relatively low levels of intraspecific genetic variability in the Hymenoptera. This paper examines predictions of some of these theories by analyzing patterns of genetic variation among the Hymenoptera, with particular reference to ants.

One theory predicts low levels of variability in organisms with haplodiploid breeding systems. In the Hymenoptera, haplodiploidy is the rule. Males are haploid and, thus, unable to exhibit heterosis; therefore, they may be expected to have lower levels of genetic variability. Crozier (1970, 1977) showed that this conclusion must be true if there is a strong correlation between male and female fitness values or if the loci in question have deleterious, recessive alleles.

A second theory which predicts lower genetic variability in Hymenoptera is based on considerations of effective population size. Effective population size is a concept which incorporates many factors, including numbers of individuals, sex-ratio, and mating system, to give an overall estimate of the size of the breeding gene pool. Crow and Kimura (1970) demonstrate that genetic variability is directly correlated with effective population size. Given either a male biased or a 1:1 sex-ratio, haplodiploid species have smaller effective population sizes than do diplodiploids with similar population sizes (Crozier, 1976, 1977). One way of increasing effective population size in populations comprised of a few eusocial colonies is to

increase the number of queens per colony. Wilson (1963) found multiple queens (polygyny) to be common among colonies of rare species of ants, where effective population size might be expected to be extremely small. If small effective population size strongly limits population variability, one might expect that polygynous species would have higher levels of genetic variability within populations than would single-queened species.

A third theory, also based on effective population size, derives its predictions from the interaction of eusociality and effective population size. Eusocial species, whether haplodiploid or diplodiploid, have smaller effective population sizes than do solitary species occurring at similar densities, because they have fewer functional reproductives per unit area. (By definition, eusocial species include substantial numbers of non-reproductives). This theory predicts that among hymenopterans, eusocial species should exhibit less genetic variability than do solitary species. Additionally, this theory predicts that polygynous species would be expected to be more variable than monogynous species due to their relatively larger effective population sizes. Since this prediction follows from effective population size considerations, the logic is the same as in the haplodiploidy based, effective population size theory.

A fourth theory is based on selectionist arguments; however, its predictions for eusocial versus solitary hymenopterans are similar to those made by the second effective population size theory. Selander and Kaufman (1973) suggest that organisms in a relatively constant environment will be less genetically variable than those for which

the environment is more variable. Eusocial species are reasonably well buffered from environmental change (particularly in physical factors) by colony structure and behavior. Due to the relatively large number of eusocial species among the Hymenoptera, especially among those hymenopterans whose population genetics has been studied, this buffering might result in generally decreased levels of genetic variability relative to other insect groups for which predominantly solitary species have been studied. This theory again predicts that among hymenopterans, eusocial species should exhibit less genetic variability than do solitary species.

In this study, I have electrophoretically investigated 3 species of dolichoderine ants. All ants are eusocial, but the number of queens per colony may vary. The three species studied were chosen to represent the spectrum from one to many queens per colony. Data from these three species are compared with those of other workers on other hymenopteran and non-hymenopteran insects. By analyzing patterns of genetic variation as a function of breeding system and social structure, predictions of theories regarding low levels of variability are tested. The roles of haplodiploidy, eusociality, and polygyny in the patterning of genetic variation are examined.

MATERIALS AND METHODS

Collections

Ants assayed in this study were collected over a thirty month period. Early collections were made from a variety of habitats including pasture, coastal sage scrub, and chaparral. These habitats spanned broadly separated geographical areas ranging from Palm Springs (Riverside Co., California) at the northern end to Punta Banda (Baja California) at the southern extreme. The east-west range was from Warner Springs (San Diego Co., California) to Upper Newport Bay (Orange Co., California). Collecting localities are indicated in Figure 1.

Large samples were taken from one habitat type, chaparral, and in one geographical area, the valley to the east of Palomar Mountain, San Diego Co. The data from six sites in this area were used to analyze the genetic structure of populations. The six Palomar sites were along California Highway 79. Each locality was separated from its nearest neighbor locality by approximately 8 km. The chaparral in this area is relatively continuous and is dominated by Adenostoma fasciculatum. Other common chaparral plants in this region are A. sparsifolium, Yucca whipplei, Rhus ovata, and Arctostaphylos spp. Collecting localities in the Palomar area are detailed in Figure 2.

The species of ants studied were Iridomyrmex pruinosum, Conomyrma insana, and Conomyrma bicolor. The results of field observations, laboratory colonies, and genetic analyses undertaken concurrently with this study strongly suggest that C. insana is monogynous, while C. bicolor and Iridomyrmex are polygynous. Polygyny in I. pruinosum

Figure 1. Collecting localities for Conomyrma insana, C. bicolor, and Iridomyrmex pruinosum. Locality symbols are as follows: N = Upper Newport Bay, Newport Beach; I = Mason Park, Irvine; L = Laguna Canyon, Laguna Beach; IN = north end of Culver Dr., Irvine; IP = Irvine Park, Orange; ET = north end of El Toro Rd., El Toro; OS = Ortega Highway, Orange Co.; O = Ortega Highway, Riverside Co.; G = Aguanga; F = Oak Grove; D = junction of California 79 and 8S10; C = Indian Flats campground; A = junction of California 79 and 9S06; B = north end of 9S02; P = Palomar Mountain Road 9S36; W = Warner Springs; PS = California 111 NW of Palm Springs; and PB = Punta Banda, Baja California, Mexico. C. insana was collected at L, IN, OS, O, G, F, D, C, A, B, P, PS, and PB. C. bicolor was collected N, I, ET, O, F, D, A, B, P, W, and PB. I. pruinosum was collected at IP, G, F, D, C, A, and B.

COLLECTING LOCALITIES

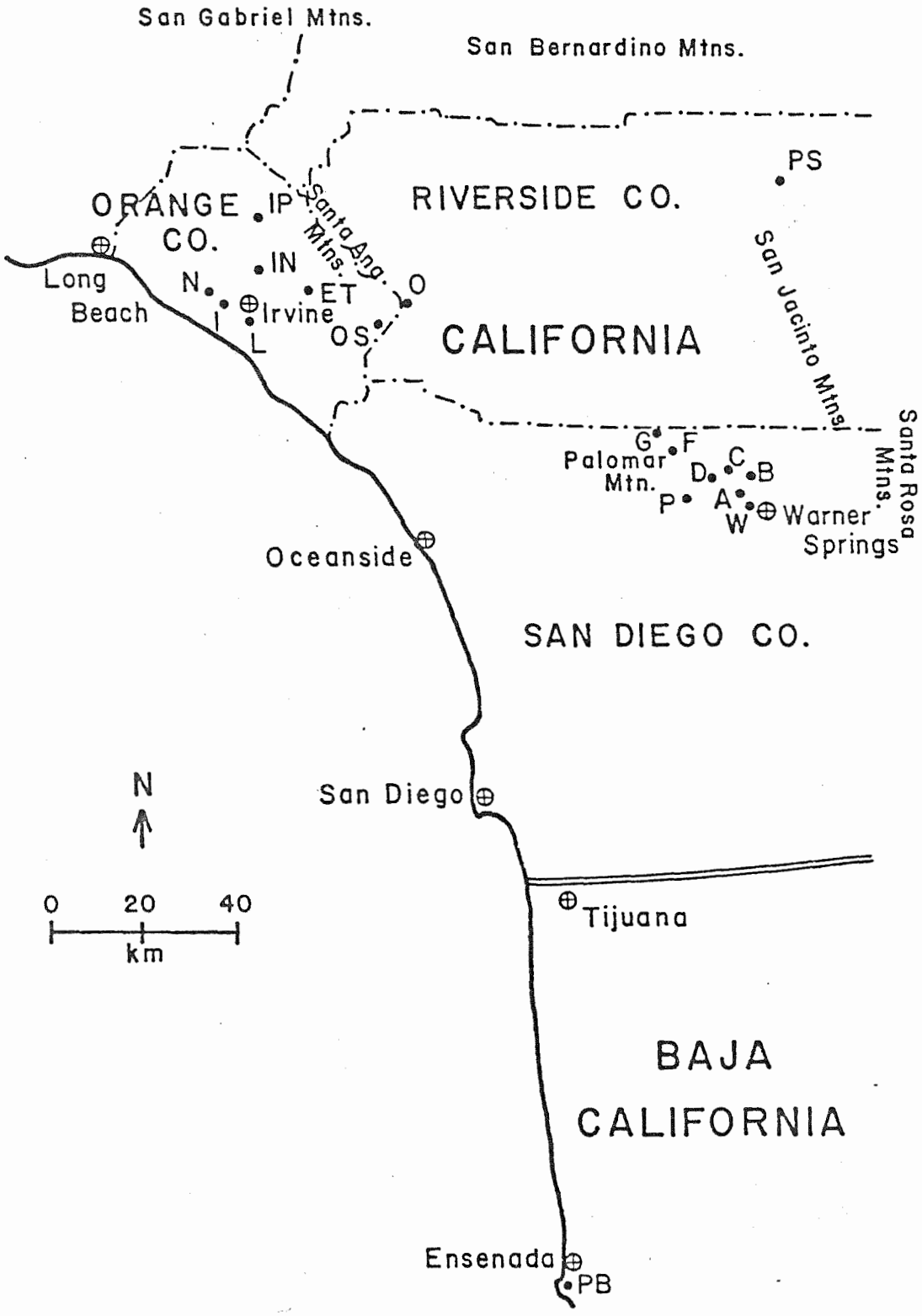
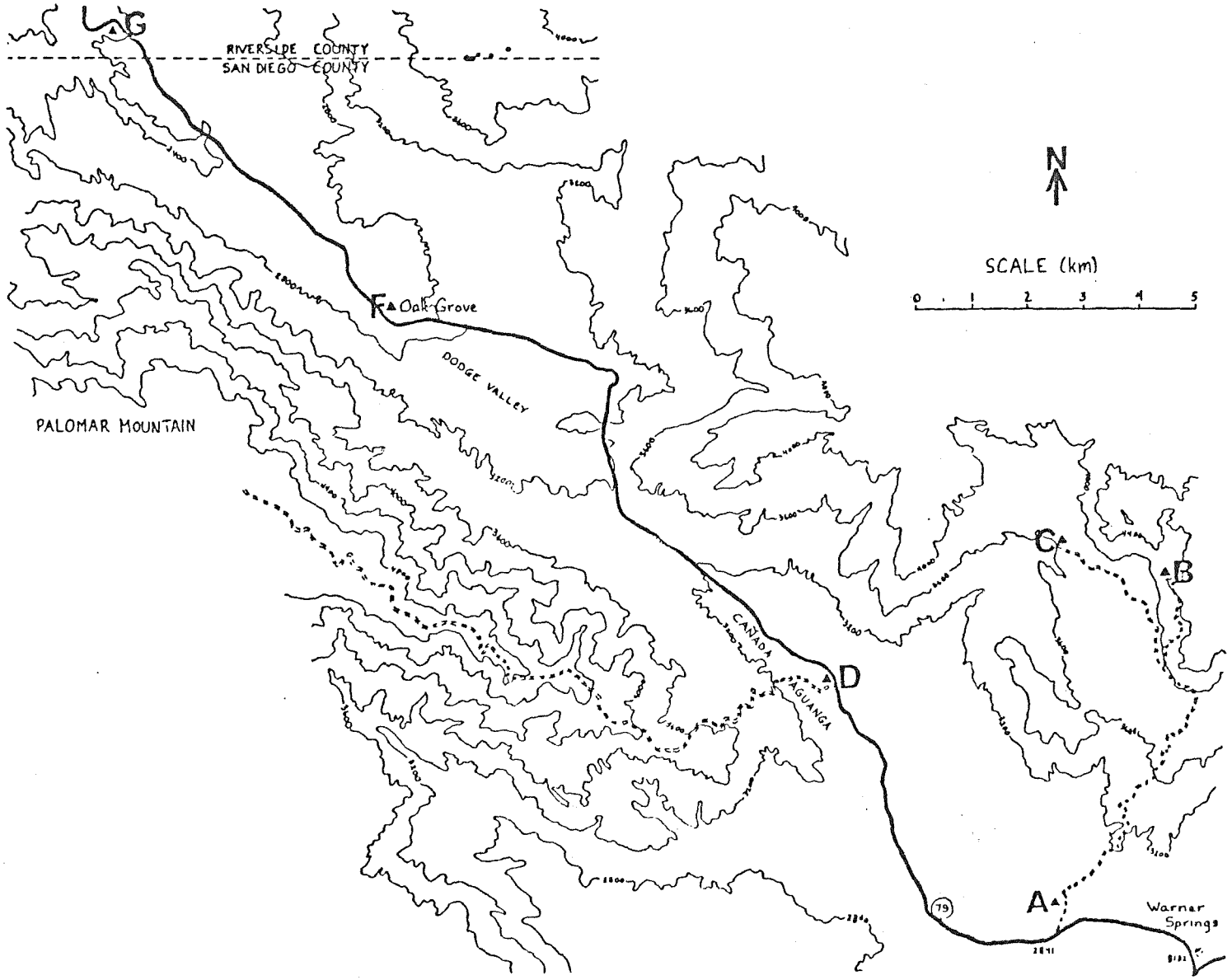


Figure 2. Collecting localities near Palomar Mountain, California. These were the localities used for population genetic analyses. Symbols are as in Figure 1.


G
RIVERSIDE COUNTY
SAN DIEGO COUNTY
F Oak Grove
DODGE VALLEY
PALOMAR MOUNTAIN
N
SCALE (km)
0 1 2 3 4 5
C
B
CAÑADA
AGUANGA
D
79
A
Warner Springs

seems to occur less frequently than in C. bicolor (Chapter 3). Sterile female workers from up to eight colonies per species per site were gathered either by digging into the soil around each nest opening or by placing a honey-water bait near the opening and then collecting ants from the bait. Collecting by baiting disturbed ants less and resulted in better survivorship. Electrophoretic patterns from relatively undisturbed ants were more distinct than from ants which had been alarmed. Whenever possible, reproductive females and males were also collected for genetic analysis. Since males are haploid, studying them in parallel with diploid females greatly aids in understanding the genetic basis underlying electrophoretic variation.

Live ants were kept cool for several hours after collection while they were transported to the laboratory where they were immediately frozen for later electrophoresis. Ants were stored at -20°C for up to 4 months. (After this time some of the systems would begin to deteriorate, as evidenced by smearing and sub-banding on gels.) Starch gel electrophoresis was performed to determine enzyme variation. Starch gel electrophoresis was selected over other electrophoretic techniques, which are known to resolve more variation, because it best lends itself to large scale comparisons of many individuals at many loci. Its widespread use by population geneticists makes comparison with the work of others relatively straightforward. Techniques employed, modified from those used by Ayala et al. (1972), are detailed below.

Gel Preparation

Horizontal starch gels were prepared using Electrostarch (Otto Hiller Electrostarch Co., Madison, Wis.). A 12.5% (w/v) solution of starch in gel buffer was heated with vigorous swirling in a large flask. The solution was brought to a hard boil and boiled for about fifteen seconds, then degassed with an aspirator and poured into a plexiglass mold, 21 x 19 x 1 cm. The gel in its mold was wrapped tightly with Handi-wrap plastic film when no longer hot to the touch, then allowed to remain at room temperature overnight. For 20 minutes immediately prior to running, gels were chilled in a refrigerator ($4^{o}C$).

Gel and Electrode Buffers

Two buffer systems were used: (A) gel buffer: 76 mM Tris and 5 mM citric acid, pH 8.65; electrode buffer: 300 mM boric acid and 60 mM NaOH, pH 8.1 (Poulik, 1957). (B) gel buffer: 9 mM Tris, 3 mM citric acid, and 1 mM EDTA, pH 7.0; electrode buffer: 135 mM Tris, 39 mM citric acid, and 1 mM EDTA. Buffers were prepared in advance and kept refrigerated for several weeks. Fresh buffer was used for each run.

Sample Preparation and Running

Ants were ground individually in 4.5 mm (diameter) wells in a 5 cm by 13.5 cm chilled teflon block. The block contained 3 rows of 9 wells so that 27 individual ants could be processed simultaneously. It was modified from a grinding block designed by Johnson (1966) for

use with Drosophila. One ant was placed in each well and .05 ml of gel buffer was added. The entire block was kept on ice during homogenization. The pestle, a short Delrin rod, 4 mm in diameter, was powered by a 3/8 inch one-speed electric hand drill (Craftsman #335.25927) mounted on a drill press (Craftsman #315.11420), run at 80 VAC. The crude homogenate was absorbed with a 10 x 4 mm piece of filter paper (Whatman #1) so that each ant provided one sample. Each sample was then blotted on a clean piece of filter paper to remove excess and inserted into a slot in the gel which had been cut with an Exacto knife. The slot was cut 5 cm from one of the ends of the gel and care was taken to make the cut clean and at a right angle to the bottom of the gel tray. After the samples had all been placed in the gel slot, the gel was sealed in the area of the slot by gentle pushing. All air bubbles were removed from between the wicks. Sometimes it was necessary to place a thin strip of sponge cloth (Amsco #240) between the gel tray and the gel end nearer the slot to ensure contact across the slot. The plastic film covering the gel was then folded so that the middle section of the gel was covered; about 4 cm of gel was exposed on the end near the slot, about 6 cm of gel was uncovered on the uncut end. The gel was placed horizontally in a buffer tank with electrodes with the slot towards the cathode. Sponge cloths (20.6 cm x 19.0 cm x 5.0 cm) were used as wicks to establish contact between the gel and the electrode buffer in the tank. They were allowed to overlap the gel slightly beyond the exposed area (about 7 cm). The gel tray and sponge cloths were weighted down by a piece of 1/8" thick glass of the same surface area as the gel tray to insure

even wick contact. A square aluminum tray filled with solidly packed crushed ice was set on top of the glass and the entire set-up was placed in a refrigerator at 4°C where it was connected to either a Heathkit (model 1P-17) or a Beckman Duostat power supply. Buffer system A gels were run for 4 1/2 hours at a constant voltage of 25 v/cm. The starting current began at about 125 mA and ended at 50 mA. Buffer system B gels were run 5 hours at a constant voltage of 20 v/cm. The current was reasonably constant and ranged between 80 and 100 mA.

After the run was completed, the two ends of the gel that were in contact with the sponge wicks were cut off and discarded. The remainder of the gel was sliced horizontally into five 2 mm thick slices using a taut wire and 2 mm thick guides. The top slice was discarded, since surface effects made it unusable. The other slices could then be used for four different enzyme assays. The order of slicing was important because assays for the least concentrated enzymes were run with the bottom slices.

Enzyme Assays

Immediately after slicing, all gels were incubated at 37°C in the dark with freshly prepared stain, with the following exceptions. Esterase and leucine amino peptidase gels were pre-soaked in 0.5 M boric acid at room temperature prior to staining. After pre-soaking, the boric acid was poured off, gels were rinsed with distilled water, stain was added, and they were incubated at 37°C in the dark. Aldehyde

oxidase gels were incubated in the dark at room temperature. Stain recipes and techniques are summarized in Table 1.

Many stains required the addition of phenazine methosulfate (PMS) after one hour to complete the reaction. The PMS was first dissolved in distilled water in the ratio of 1 mg PMS to 1 ml water, then the required amount of PMS solution was added to the stain box.

Esterase and glutamate oxaloacetate transaminase and aldehyde oxidase stains developed within one hour. Phosphoglucomutase, phosphoglucose isomerase and malic dehydrogenase developed within one-half to one hour after the addition of PMS. Other stains took up to 6 hours. All stains were checked periodically and removed from the incubator when bands appeared readable.

Gel Fixation

After the enzyme bands appeared, the reaction was stopped by aspirating the stain into a trap, rinsing the gel with several rinses of distilled water and then replacing the final wash with a fixing solution: 5 parts methanol to 5 parts water to 1 part acetic acid. All clearly distinguishable bands were scored either prior to fixation or shortly after fixation and prior to wrapping. Fixation changed the consistency of the gels, but their readability was not impaired for a few days. Many stains remained readable for months. After 8 hours in fixing solution, gels were wrapped and sealed airtight with Saran Wrap for long-term storage at room temperature in the dark.

The abbreviations used to designate each enzyme are indicated in Table 1. These same abbreviations, where written in upper case

Table 1. Enzyme assay techniques.

Enzyme	Run Buffer	Stain	Stain Buffer	Special Instructions
Aldehyde oxidase (Ao)	A	2.5 mg PMS 10 mg NBT 12.5 mg NAD 5 mg EDTA 5 ml Benzaldehyde	50 ml .06 M Tris-HCl pH 8.6	Incubate in dark at room temperature
Esterase (Est)	A	.75 ml 2% β-naphthyl-acetate solution (1:1, water: acetone) 30 mg Fast Garnet GBC salt	50 ml 0.1 M Phosphate pH 6.5	Pre-soak in .5 M Boric acid; 15 minutes Incubate at 37°C
Glutamate-oxaloacetate-transaminase (Got)	A	75 mg Fast Blue BB salt 5 mg Pyridoxal 5' phosphate 100 mg Aspartic acid 50 mg α-ketoglutaric acid	50 ml .05 M Tris-HCl pH 8.0	Incubate at 37°C
Hexokinase (Hk)	B	10 mg NBT 12.5 mg TPN 12.5 mg ATP 10 mg $MgCl_2$ 400 mg Glucose 40 units Glucose-6-phosphate dehydrogenase	50 ml 0.1 M Tris-HCl pH 7.1	Incubate at 37°C After 1 hour add 2.5 mg PMS
Isocitrate dehydrogenase (Idh)	B	10 mg NBT 10 mg TPN 100 mg Sodium isocitrate 90 mg $MgCl_2$	50 ml 0.1 M Tris-HCl pH 7.1	Incubate at 37°C After 1 hour add 2.5 mg PMS

Table 1. (cont.)

Enzyme	Run Buffer	Stain	Stain Buffer	Special Instructions
Leucine amino peptidase (Lap)	A	35 mg L-leucyl-β-naphthyl-amide HCl 15 mg Black K salt	25 ml 0.2 M maleic anhydride, 0.2 M NaOH 5 ml 0.2 M NaOH 20 ml H_2O (pH 5.0)	Pre-soak in .5 M Boric acid; 20 minutes Incubate at 37°C
Lactate dehydrogenase (Ldh)	B	10 mg NBT 12.5 mg NAD 5 ml 8% DL-lactate in 0.5 M Na_2CO_3	50 ml 0.1 M Tris-HCl pH 7.1	Incubate at 37°C After 1 hour add 2.5 mg PMS
Malic dehydrogenase (Mdh)	B	10 mg NBT 12.5 mg NAD 25 mg L-malic acid	50 ml .06 M Tris-HCl pH 7.1	Incubate at 37°C After 1 hour add 2.5 mg PMS
Malic enzyme (Me)	B	10 mg NBT 10 mg TPN 12.5 mg $MgCl_2$ 25 mg L-malic acid	50 ml .06 M Tris-HCl pH 7.1	Incubate at 37°C After 1 hour add 2.5 mg PMS
Phosphoglucose isomerase (Pgi)	B	10 mg NBT 5 mg TPN 100 mg $MgCl_2$ 10 mg Fructose-6-phosphate 12.5 mg EDTA 40 units Glucose-6-phosphate dehydrogenase	50 ml 0.1 M Tris-HCl pH 7.1	Incubate at 37°C After 1 hour add 2.5 mg PMS

Table 1. (cont.)

Enzyme	Run Buffer	Stain	Stain Buffer	Special Instructions
Phosphogluco-mutase (Pgm)	B	10 mg NBT 5 mg TPN 100 mg $MgCl_2$ 300 mg Glucose-1-phosphate 40 units Glucose-6-phosphate dehydrogenase	50 ml 0.1 M Tris-HCl pH 7.1	Incubate at 37°C After 1 hour add 2.5 mg PMS
Hydroxybutyrate dehydrogenase (Hbdh)	B	10 mg NBT 25 mg NAD 5 mg $MgCl_2$ 288 mg NaCl 630 mg DL-hydroxybutyrate	15 ml 0.5 M Tris-HCl pH 7.1 35 ml H_2O	Incubate at 37°C After 1 hour add 2.5 mg PMS
Indophenal oxidase (Ipo)	B	Any stain which uses NBT and PMS	See stain being used	Shows up as a clear band after several hours staining

NBT = Nitro blue tetrazolium
NAD = β-nicotinamide adenine dinucleotide
TPN = Nicotinamide adenine dinucleotide phosphate
ATP = Adenosine triphosphate
PMS = Phenazine methosulfate

letters, are used to represent the genes coding for the enzymes. When several forms of the same enzyme exist, each controlled by a different gene locus, a hyphenated number is attached to the enzyme symbol to designate the enzyme system. The isozyme system that migrates most cathodally or least anodally is given the lowest number; the most anodal is given the highest number. For the enzymes studied, the alleles are numbered by superscripts. Again, lower numbers represent more cathodal or less anodal migrants. Thus GOT-2^1 and GOT-2^2 represent, respectively, the "slow" and the "fast" alleles at the GOT-2 locus. The GOT-2 system migrates more anodally than does GOT-1, which actually migrates cathodally under the running conditions studied.

RESULTS

Data are presented from sixteen enzyme systems in Conomyrma insana, thirteen in Conomyrma bicolor, and thirteen in Iridomymex pruinosum. Many more systems were surveyed but were not suitable for further study because their appearance on gels was either too faint or too unreliable. Tables 2-4 summarize the allele frequencies for those loci studied in each species. These data are from a minimum of six individuals from each of at least two different colonies, except as indicated. In most cases, sample sizes are much larger. Figures drawn from photographs of representative gels of variable systems for each species are included in the Appendix.

Geographic Variation

Allele frequencies were analyzed along a broad geographic transect from Irvine at the northwestern extreme to Warner Springs at the southeastern end. GOT-2 was a highly polymorphic system in Conomyrma bicolor at all localities. Allele frequencies are enumerated in Table 5. A Kruskal-Wallis one-way ANOVA indicates that these data do not come from one population ($p < .01$). When arcsine transformed frequencies of the GOT-2^1 allele are plotted as a function of distance from Irvine in km (Figure 3), a highly significant relationship between allele frequency and position along the transect is evident ($p \ll .001$, regression coefficient $\beta = .29$). Within the Palomar area (sites A, B, D, and F), GOT-2^1 frequencies do not differ among sites (Kruskal-Wallis one-way ANOVA, $p > .1$).

Table 2. Allele frequencies at 16 enzyme loci in populations of Conomyrma insana.

Allele or Allozyme‡		Population B'	L	O	P	PB	PS	A	B	C	D	F	G
	n:	2	2	2	2	5	2	5	10	5	7	4	4
LAP[1]**		0.00	0.06	0.00	0.00	0.00	0.00	0.00*	0.00*	0.00*	0.00*	-	-
LAP[2]**		1.00	0.94	1.00	1.00	1.00	1.00	1.00*	1.00*	1.00*	1.00*	-	-
AO[1]		1.00	0.00	1.00	1.00	1.00	0.00*	-	-	-	-	-	-
AO[2]		0.00	1.00	0.00	0.00	0.00	1.00*	-	-	-	-	-	-
MDH-2[1]		0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	0.00	0.06	0.00
MDH-2[2]		1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	0.94	1.00
PGM[1]		0.00	0.00	-	-	-	0.04	0.00	0.00	0.00	0.06	0.00*	0.00
PGM[2]		0.90	0.21	-	-	-	0.96	1.00	1.00	0.67	0.94	1.00*	1.00
PGM[3]		0.10	0.79	-	-	-	0.00	0.00	0.00	0.33	0.00	0.00*	0.00
PGI[1]		0.77	1.00	1.00	1.00	1.00	0.50	0.98	0.83	0.89	0.92	1.00	1.00
PGI[2]		0.23	0.00	0.00	0.00	0.00	0.50	0.02	0.17	0.01	0.00	0.00	0.00
PGI[3]			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.08	0.00	0.00
HK		-	-	-	-	1.00	-	-	-	-	-	-	-

Table 2. (cont.)

Allele‡	B'	L	O	P	PB	PS	A	B	C	D	F	G
IDH^1	1.00	1.00	1.00	-	1.00	1.00	1.00*	1.00	1.00	0.94	0.89	1.00
IDH^2	0.00	0.00	0.00	-	0.00	0.00	0.00*	0.00	0.00	0.06	0.11	0.00
EST-1$^+$**	-	1.00	1.00	-	1.00	-	1.00*	1.00*	1.00*	0.75	-	-
EST-1-**	-	0.00	0.00	-	0.00	-	0.00*	0.00*	0.00*	0.25	-	-
ME^1**	0.00	0.00	0.00	-	0.00	0.00	-	0.00	0.00	0.00	0.10	0.00
ME^2**	1.00	1.00	1.00	-	1.00	1.00	-	1.00	1.00	1.00	0.90	1.00
GOT-2^1**	1.00	1.00	1.00	-	1.00	-	0.88*	0.93*	1.00*	1.00	-	-
GOT-2^2**	0.00	0.00	0.00	-	0.00	-	0.12*	0.07*	0.00*	0.00	-	-
HBDH	1.00	-	-	-	-	1.00	-	-	-	-	-	-
MDH-1	1.00	1.00	-	-	-	1.00	-	1.00	1.00*	1.00	1.00*	-
GOT-1	-	1.00	1.00	-	-	-	-	1.00*	1.00*	1.00*	-	-
EST-6	-	-	-	-	-	-	1.00*	1.00*	1.00*	1.00	-	-

Table 2. (cont.)

Allele‡	B'	L	O	P	PB	PS	A	B	C	D	F	G
LDH	-	-	-	-	-	-	1.00*	1.00	1.00	1.00	1.00	1.00*
IPO-2	-	-	-	-	-	-	-	1.00*	1.00	1.00*	1.00*	1.00*

‡ For some enzyme systems, resolution or data were insufficient to allow a genetic interpretation of the variability present. For these systems, allozyme frequencies are given instead of allele frequencies.

n Number of colonies sampled in the population; population symbols are as in Figure 1; B' is an early sample very near B.

** Allozyme frequencies follow in place of allele frequencies for this system. In the case of EST-1, the band was either present or absent. Presence of the band is indicated by allozyme EST-1^{+}, absence by EST-1^{-}.

* These data represent frequencies from only one colony.

Table 3. Allele frequencies at 13 enzyme loci in populations of Conomyrma bicolor.

Allele or Allozyme‡	Population										
	I1	I2	I3	O	B'	W	PB	A	B	D	F
n:	7	4	3	3	2	4	2	4	9	8	3
MDH-2	1.00	1.00	1.00	1.00	1.00*	1.00	1.00	1.00	1.00	1.00	1.00
AO^1**	1.00	1.00	-	1.00*	0.70*	0.75	1.00	0.58	0.88	0.61	0.76
AO^2**	0.00	0.00	-	0.00*	0.30*	0.25	0.00	0.42	0.12	0.39	0.24
GOT-2^1	0.33	0.18	0.00*	0.40	0.81	0.81	-	0.57	0.82	0.75	0.58
GOT-2^2	0.67	0.82	1.00*	0.60	0.19	0.19	-	0.43	0.18	0.25	0.42
EST-1^+**	0.33	0.06	0.00	0.00	0.48	0.31	1.00	0.45	0.36	0.40	-
EST-1^-**	0.67	0.94	1.00	1.00	0.62	0.69	0.00	0.55	0.64	0.60	-
LAP	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IDH^1	0.00	0.16	0.00	0.00	0.00	0.00*	0.00*	0.00	0.00	0.02	-
IDH^2	1.00	0.84	1.00	0.80	1.00	1.00*	1.00*	1.00	1.00	0.98	-
IDH^3	0.00	0.00	0.00	0.20	0.00	0.00*	0.00*	0.00	0.00	0.00	-

Table 3. (cont.)

Allele‡	I1	I2	I3	O	B'	W	PB	A	B	D	F
IPO-2	1.00	1.00	-	1.00	1.00	1.00	1.00*	1.00	1.00	1.00	-
LDH[1]**	1.00	1.00*	-	1.00	1.00*	1.00	1.00*	1.00	0.78	0.86	1.00
LDH[2]**	0.00	0.00*	-	0.00	0.00*	0.00	0.00*	0.00	0.22	0.14	0.00
ME	1.00	1.00*	1.00	1.00	1.00*	1.00	1.00	1.00	1.00	1.00	-
PGI[1]	-	0.89	-	0.50	0.39*	0.39	-	0.62	0.61	0.53	0.54
PGI[2]	-	0.11	-	0.50	0.61*	0.61	-	0.38	0.39	0.47	0.46
HK-2	-	1.00	-	1.00	-	1.00	1.00*	1.00	1.00	1.00	-
EST-6[1]**	-	-	-	-	-	-	-	1.00	0.99	1.00	1.00
EST-6[2]**	-	-	-	-	-	-	-	0.00	0.01	0.00	0.00
GOT-1	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00

‡ For systems that had insufficient data for a genetic interpretation, allozyme frequencies are given.
n Number of colonies sampled; population symbols are as in Figure 1 and Table 2.
** Allozyme frequencies follow instead of allele frequencies. EST-1 is as in Table 2.
* These data represent frequencies from only one colony.

Table 4. Allele frequencies at 13 enzyme loci in populations of Iridomyrmex pruinosum.

Allele or Allozyme‡	Population							
	IP	B'	A	B	C	D	F	G
n:	3	10	8	1	4	6	3	5
IDH[1]	0.38	1.00	0.94	1.00*	1.00	0.99	1.00	0.96
IDH[2]	0.62	0.00	0.06	0.00*	0.00	0.00	0.00	0.04
IDH[3]	0.00	0.00	0.00	0.00*	0.00	0.01	0.00	0.00
PGI[1]	0.00	0.02	0.00	0.06*	0.00	0.00	0.00	0.00
PGI[2]	1.00	0.98	1.00	0.94*	1.00	1.00	1.00	1.00
PGM[1]	1.00	1.00	0.61	1.00*	1.00	0.64	0.64	1.00
PGM[2]	0.00	0.00	0.39	0.00*	0.00	0.36	0.36	0.00
ME[1]**	0.28	0.07	0.08	0.20*	0.31	0.15	0.00	0.16
ME[2]**	0.42	0.06	0.92	0.80*	0.69	0.85	1.00	0.75
ME[3]**	0.30	0.87	0.00	0.00*	0.00	0.00	0.00	0.09
MDH-1[1]	0.45	0.92	-	1.00*	-	-	-	-
MDH-1[2]	0.55	0.08	-	0.00*	-	-	-	-
MDH-2[1]	0.00	0.03	0.00	-	0.00	0.00	0.00	0.10
MDH-2[2]	0.10	0.96	1.00	-	1.00	1.00	1.00	0.90
MDH-2[3]	0.00	0.01	0.00	-	0.00	0.00	0.00	0.00
GOT-1	-	1.00	1.00	-	1.00*	1.00	-	-
AO	-	1.00	1.00	-	1.00*	1.00	-	-
αGPDH-1	-	-	1.00	-	1.00*	1.00	-	-
αGPDH-2	-	-	1.00	-	1.00*	1.00	-	-

Table 4. (cont.)

Allele	IP	B'	A	B	C	D	F	G
GOT-2	-	-	1.00	-	1.00*	1.00	-	-
IPO	-	-	1.00	-	1.00*	1.00	-	-
HK	-	-	1.00	-	1.00*	1.00	-	-

‡ For systems that had insufficient data for a genetic interpretation, allozyme frequencies are given.
n Number of colonies sampled; population symbols are as in Figure 1 and Table 2.
** Allozyme frequencies follow instead of allele frequencies.
* These data represent frequencies from only one colony.

Table 5. Frequencies of GOT-2 alleles in Conomyrma bicolor colonies.

		Workers			Males		
Locality*	Colony	GOT-2^1	GOT-2^2	n	GOT-2^1	GOT-2^2	n
I1	1	0.27	0.73	26			
	2	0.48	0.52	20			
	3	0.50	0.50	12			
	4	0.27	0.73	145			
	5	0.08	0.92	6			
	6	0.40	0.60	15			
I2	1	0.25	0.75	6			
	2	0.12	0.88	20			
	3	0.22	0.78	64	0.03	0.97	64
	4	0.12	0.88	8	0.43	0.57	14
I3	1	0.00	1.00	8			
ET	1	0.42	0.58	31			
O	1	0.31	0.69	42	0.38	0.62	8
	2	0.48	0.52	43	0.67	0.33	3
P	1	0.71	0.29	7	1.00	0.00	2
W	1	0.92	0.08	60			
	2	0.69	0.31	68			
	3	0.82	0.18	14			
A	1	1.00	0.00	16			
	2	0.34	0.66	32			
	3	0.44	0.56	32			
	4	0.48	0.52	24			

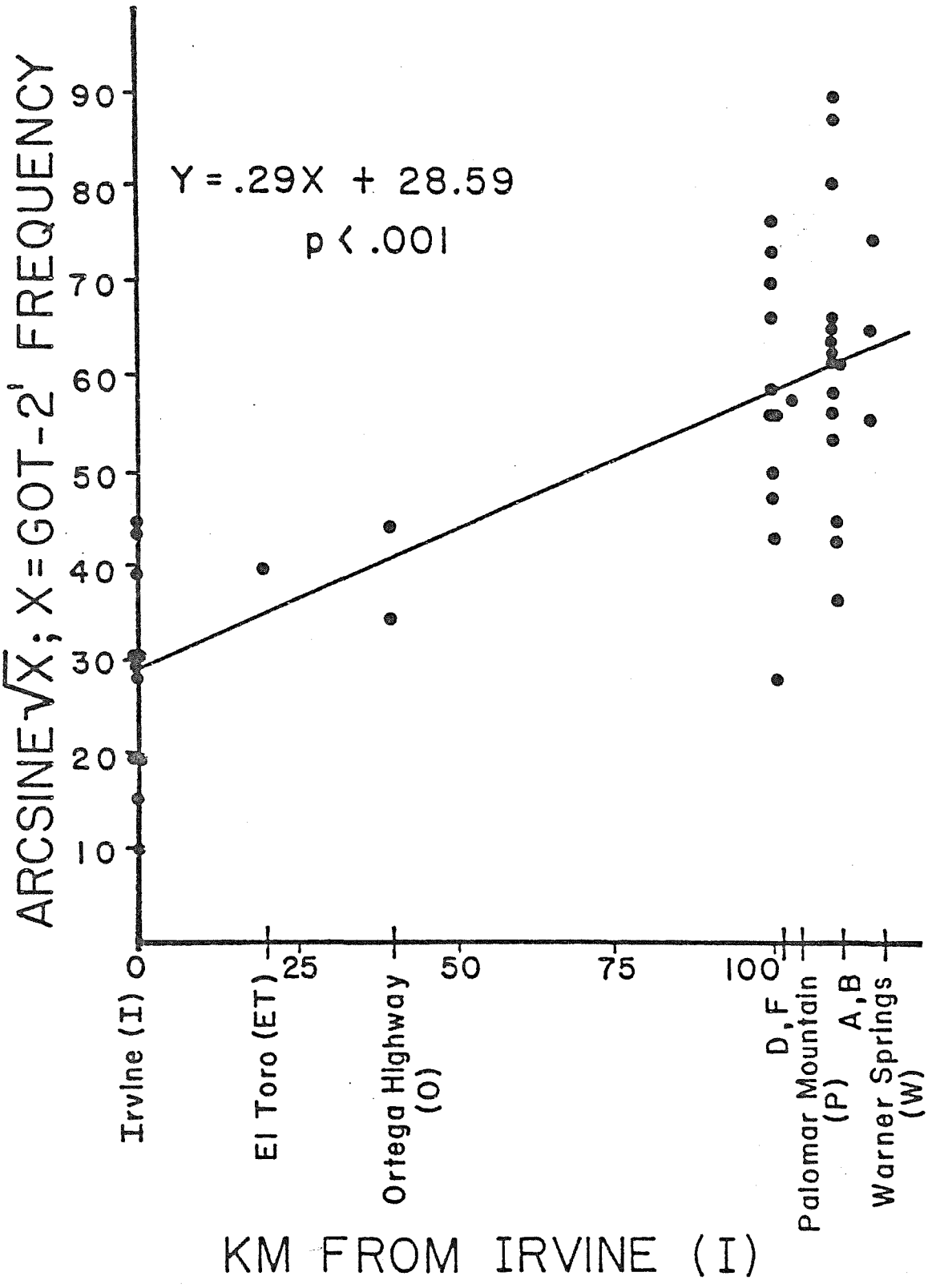
Table 5. (cont.)

Locality*	Colony	Workers			Males		
		GOT-2^1	GOT-2^2	n	GOT-2^1	GOT-2^2	n
B	1	0.84	0.16	32	0.62	0.38	13
	2	1.00	0.00	8			
	3	0.79	0.21	24			
	4	0.78	0.22	16			
	5	0.69	0.31	24			
	C5	1.00	0.00	8			
	6	0.78	0.22	16			
	7	0.81	0.19	13			
	8	0.72	0.28	16			
	11	0.82	0.18	40			
	12	0.63	0.37	23			
D	1	0.81	0.19	16			
	2	0.22	0.78	16			
	3	0.91	0.09	16			
	4	0.69	0.31	16			
	5	0.94	0.06	16			
	6	0.84	0.16	16			
	7	0.88	0.12	16			
	8	0.72	0.28	16			
F	1	0.47	0.53	32			
	2	0.69	0.31	31			
	3	0.58	0.42	32			

* See Figure 1 for locations of collecting localities. I1, I2, and I3 are three sites at locality I that are separated by about .5 km.

Figure 3. GOT-2^1 frequencies along a transect from Irvine, California to Warner Springs, California. Arcsine transformed allele frequencies ($\sin^{-1} z^{1/2}$, z = frequency of GOT-2^1) are plotted as a function of distance (km) from Irvine along a line connecting Irvine and Warner Springs.

GOT-2' FREQUENCIES ALONG A TRANSECT



The clinal variation at the GOT-2 locus in Conomyrma bicolor across Southern California may be due either to limited gene flow along the axis studied and (or) varying adaptive regimes. In Pogonomyrmex barbatus, Johnson et al. (1969) showed that allelic frequencies at three enzyme loci were correlated with a combination of rainfall, temperature and elevation. Since I wanted to examine genetic variation of populations as a function of social structure, as opposed to as a function of geographic variation, detailed analyses of variation have been confined to six Palomar area sited that were demonstrated to be homogeneous at the GOT-2 locus.

Genetic Variability

For each of the three species studied, two widely used measures, P and H, which reflect the level of genetic variability within populations, were calculated for the six Palomar populations. To calculate P, the average percent of loci polymorphic per population, all loci listed in Tables 2-4 were used. A locus was considered polymorphic in a population if the most common allozyme had a frequency less than or equal to 0.99. Heterozygosity for a locus is defined as $1 - \Sigma X_i^2$, where X_i is the frequency of the i^{th} allele and mean heterozygosity (H) is the mean over all loci examined. Mean heterozygosity values were calculated in two ways. One estimate (H^*) included only those loci for which the genetic basis of allozyme variation was easily inferred. The second estimate (H) was an average over all loci. Assumptions about the genetic basis of variation were made as detailed in the Appendix. Both of these estimates and P values for all three

species are listed in Table 6. It is expected that $\underline{H}$ will be greater than $\underline{H}^*$ since some variable loci are eliminated from the estimate of the latter.

Table 6. Genetic variation in three species of ants.

Species	P	H ± s.d.	H* ± s.d.
Conomyrma insana	21%	0.040 ± .022	0.035 ± .025
Conomyrma bicolor	39%	0.130 ± .010	0.102 ± .010
Iridomyrmex pruinosum	24%	0.052 ± .039	0.039 ± .026

P, H, and H* See text for explanation.

DISCUSSION

Heterozygosity values for the species reported here are compared in Table 7 with those obtained for populations of a variety of distantly related insect species and for all other Hymenoptera for which data are available. The data cited from this study are the H^* values (see Results), since H values may include non-genetic or uncertain amounts of genetic variation. By making comparisons at the appropriate level, it is possible to examine the roles of polygyny, eusociality and haplodiploidy in determining the level of genetic variability exhibited by the species studied.

Polygyny and Genetic Variation

Some workers (e.g. Pamilo et al., 1978b) have partially ascribed low levels of variability within the Hymenoptera to the preponderance of small deme size. One way in which effective population size may be increased among eusocial Hymenoptera is by polygyny (Wilson, 1963). One of the polygynous species studied, *Conomyrma bicolor*, did have relatively high levels of genic variation, the highest reported for any hymenopteran studied to date. The second polygynous species studied, *Iridomyrmex pruinosum*, however, had relatively low mean heterozygosity. This finding is not inconsistent with the predictions about polygyny if we note that a genetic analysis of mating systems (Chapter 3) reveals that polygyny is probably much less common in *I. pruinosum* than in *C. bicolor*. It should be noted that individuals of *I. pruinosum* were the smallest studied and the number of enzymes

Table 7. Level of genetic variation in insect populations. Symbols following hymenopteran species names are as follows: S = solitary, PE = primitively eusocial, E = eusocial, W = wasp, B = bee, A = ant, SF = sawfly, P = polygynous, O = oligogynous, and M = monogynous. (All ants are eusocial.)

Organism	H	Number of populations	Mean Number of loci	Reference
ORTHOPTERA				
Gryllus integer	.145	-	20	Selander & Johnson, 1972
*Ceuthophilus gracilipes**	.026	3	26	Cockley et al., 1977
HOMOPTERA				
Magicicada tredecassini	.174	2	15	Krepp & Smith, 1974
M. tredecula	.153	2	15	"
Heliothis virescens	.278	12	13	Sluss et al., 1978; Sluss & Graham, 1979
H. zea	.270			"
Philaenus spumarius	.076	6	23	Saura et al., 1973
HETEROPTERA				
Gerris lacustris	.062	9	19	Varvio-Aho et al., 1978
G. odontogaster	.252**	2	8	"
G. argentatus	.224**	1	8	"

* Cave-dwelling species
** Very small samples; all loci sampled polymorphic

Table 7. (cont.)

Organism	H	Number of populations	Mean Number of loci	Reference
COLEOPTERA				
Otiorrhynchus scaber	.309	1	24	Suomalainen & Saura, 1973
Strophosomus capitatus	.157	3	19	"
*Ptomaphagus hirtus**	.048	6	13	Laing et al., 1976
LEPIDOPTERA				
Colias alexandra	.146	2	13	G.B. Johnson (pers. comm. in Powell, 1975)
C. meadii	.160	1	12	"
C. philodice	.200	1	12	"
Solenobia triquetrella	.182	2	16	Lokki et al., 1975
DIPTERA				
Drosophila willistoni	.198	94	35	summarized from Powell, 1975
D. melanogaster	.154	25	21	"
D. simulans	.107	3	20	"
D. obscura	.109	57	30	"
D. subobscura	.105	30	34	"

* Cave-dwelling species

Table 7. (cont.)

Organism	H	Number of populations	Mean Number of loci	Reference
DIPTERA (cont.)				
Drosophila spp.***	.175	429	16	summarized from Powell, 1975
HYMENOPTERA				
Stictia carolina (S,W)	.056	1	17	Metcalf et al., 1975
Chalybion californicum (S,W)	.073	1	16	"
Sceliphron caementarium (S,W)	.078	1	12	"
Scolia dubia dubia (S,W)	.051	1	15	"
Trypargilum politum (S,W)	.059	1	19	"
Nomia heteropoda (S,B)	.070	1	15	"
Savastra obliqua (S,B)	.038	1	16	"
Augochlora pura (S,B)	.000	-	24	Snyder, 1974
Lasioglossum zephyrum (PE,B)	.000	-	13	"
Bombus americanorum (PE,B)	.000	-	12	"
Opius juglandis (S,W)	.043	6	13	Lester & Selander, 1979

*** Pooled value for 29 species

Table 7. (cont.)

Organism	H	Number of populations	Mean Number of loci	Reference
HYMENOPTERA (cont.)				
Megachile pacifica (S,B)	.033	5	17	Lester and Selander, 1979
Nomia melanderi (S,B)	.041	3	13	"
Polistes exclamans (E,W)	.040	8	16	"
P. annularis (E,W)	.053	1	15	"
P. apachus (E,W)	.084	3	13	"
P. bellicosus (E,W)	.071	3	13	"
Apis mellifera (E,B)	.010	4	39	Sylvester, 1976
A. mellifera (E,B)	.012	1	16	Pamilo et al., 1978b
Bombus lucorum (PE,B)	.010	3	16	"
B. terrestris (PE,B)	.037	1	15	"
B. hypnorum (PE,B)	.048	1	12	"
B. lapidarius (PE,B)	.007	1	16	"
B. pascuorum (PE,B)	.001	3	10	"
B. hortorum (PE,B)	.000	1	11	"

Table 7. (cont.)

Organism	H	Number of populations	Mean Number of loci	Reference
HYMENOPTERA (cont.)				
Macropis labiata (S,B)	.033	2	10	Pamilo et al., 1978b
Colletes succincta (S,B)	.064	2	8	"
Andrena clarkella (S,B)	.037	1	13	"
A. lapponica (S,B)	.007	1	10	"
A. vaga (S,B)	.000	1	9	"
Vespula vulgaris (PE,W)	.000	1	13	"
Mimesa equestris (S,W)	.000	1	10	"
Pontania vesicator (S,SF)	.021	1	18	"
Rhitidoponera impressa (A)	.036	35	22	P. Ward, 1978
Iridomyrmex purpureus (A)	.030	4	20	Halliday, 1978
Formica aquilonia (A,P)	.049	3	13	Pamilo et al., 1978a
F. polyctena (A,P)	.020	3	14	"
F. uralensis (A,P)	.000	2	12	"

Table 7. (cont.)

Organism	H	Number of populations	Mean Number of loci	Reference
HYMENOPTERA (cont.)				
F. lugubris (A,O)	.044	3	13	Pamilo et al., 1978a
F. exsecta (A,O)	.047	3	14	"
F. cinerea (A,O)	.062	1	9	"
F. sanguinea (A,O)	.078	3	12	"
F. rufa (A,M)	.049	4	14	"
F. pratensis (A,M)	.048	1	12	"
F. fusca (A,M)	.050	3	10	"
F. transkaucasica (A,M)	.068	2	10	"
F. rufibarbis (A,M)	.019	1	10	"
F. truncorum (A,M or O)	.017	1	12	"
Conomyrma insana (A,M)	.035	6	8	This study
C. bicolor (A, O or P)	.102	4	9	"
Iridomyrmex pruinosum (A,P)	.039	6	8	"

scanned for variability was fewer (15) than for the other species studied (the mean number of loci scanned was 22.5 for C. bicolor and C. insana). This was because only 15 enzyme systems could be visualized, and many of these were too faint for consistent scoring. Therefore, perhaps technical complications contributed to the low variability seen in I. pruinosum.

Among all ant species studied, the hypothesis that polygyny results in increased levels of genetic variation within populations was tested. An analysis of the data on 6 monogynous and 9 polygynous or oligogynous species (Table 7) does not support the hypothesis. Heterozygosity values among the two groups do not differ significantly (Mann-Whitney U test, one-tailed $p >> .10$). Although Conomyrma bicolor shows relatively high levels of variability, polygynous and oligogynous ants as a group do not. More data on a taxonomically diverse array of species are needed to elucidate general patterns, if they exist, correlating the level of population genetic variation and the number of queens per colony.

Eusociality and Genetic Variation

Levels of allozyme variation for the three species of ants studied within the Palomar area are low relative to those found for other insect groups, but are consistent with previous data on Hymenoptera. Two bases for low levels of heterozygosity among the Hymenoptera have been pointed out by other workers: (1) the relatively large number of eusocial, colonial species, and (2) their haplodiploid breeding systems.

Although haplodiploidy and eusociality have been tightly linked in sociobiological theory (Hamilton, 1964; Wilson, 1971), they may represent independent attributes among the Hymenoptera. For example, one possibility is that they are both responses to highly inbred breeding systems common among Hymenoptera. Eusociality is found in about 10% of hymenopteran species and is thought to have evolved independently at least 11 times (Wilson, 1966). Both the physical and behavioral aspects of colony structure in eusocial species have been demonstrated to provide considerable homeostasis with respect to environmental variation. Temperature, humidity, nitrogen balance, oviposition rate, and foraging intensity have all been shown to be relatively finely tuned in some eusocial species (Wilson, 1971). Colony structure may also result in increased longevity of reproductives. Queen ants may live in captivity as long as 10 years and are among the longest lived of all insects (Bodenheimer, 1937; Wilson, 1971).

Selander and Kaufman (1973) have proposed an extension of Levins' ideas (1968) on environmental grain to explain differences in levels of variability among taxa. They suggest that relatively large, long-lived organisms experience the world in a fine-grained manner and rely on behavioral and physiological adaptation to environmental fluctuation. Smaller, more short-lived animals, they propose, see the world as more coarse-grained and may spend their entire lives in one kind of habitat patch. To deal with long-term and large-scale variation between habitat patches, they might be expected to rely on genetically variable offspring. By this argument, small, short-lived organisms might be expected to have higher levels of population genetic variability.

This theory predicts that among the Hymenoptera, eusocial species should be less variable than are solitary species and explains the low level of variability among Hymenoptera relative to other insects as due to the large number of eusocial species studied.

To test the hypothesis that eusociality is correlated with low levels of genetic variability among Hymenoptera, $\underline{H}$ values for eusocial, primitively eusocial, and solitary species were compared. Nine primitively eusocial species show significantly less variation than either the 23 eusocial species (Mann-Whitney U test, one-tailed $p < .0005$) or the 18 solitary species studied (Mann-Whitney U test, one-tailed $p < .01$). Solitary and eusocial species show similar patterns of genetic variability (Mann-Whitney U test, one-tailed $p = .2358$). When the data are analyzed separately for bees and wasps, the trends are the same.

Pamilo et al. (1978b) found a non-significant trend in the predicted direction. However, my reanalysis of their data, along with all available data on Hymenoptera, shows there to be no such trend. The only general pattern which emerges is that populations of primitively eusocial species are considerably less variable than are those of either more advanced eusocial species or solitary hymenopterans. This finding is not consistent with the predictions of Selander and Kaufman's theory. However, it is compatible with the idea that eusociality arises in species with relatively inbred populations. Primitively eusocial species may have population genetic structures more similar to those of species in the early stages of becoming eusocial. Increased genic variation among species with more

complex societies may not result in breakdown of the social structure if behavior in these species is more canalized and if the social structure has thrust the species into a new adaptive zone (sensu Waddington) where it is extremely successful. Any speculation on this topic, however, should be made cautiously because seven of the nine primitively eusocial species belong to the genus Bombus which may have reduced variability for historical reasons. Once again, the need for taxonomically diverse sources of data is apparent.

Haplodiploidy and Genetic Variation

Both selectionist and neutralist theories predict that haplodiploid organisms should have lower genetic variability than diplodiploids. One important parameter in all of these theories is effective population size. Genetic diversity within a population is expected to be lower when effective population size is lower. In the absence of female biased sex-ratios, species with male haploidy have relatively lower effective population sizes than do those with male diploidy (Crozier, 1976, 1977). Although ratios of investment in the sexes may be female biased among many eusocial hymenopterans (Trivers and Hare, 1976), the actual sex-ratio of numbers of reproductives is often male biased (e.g. Scherba, 1961; Plateaux-Quénu, 1962; Berkelhamer, unpubl.).

Data on mean heterozygosities per population (H, Table 7), have been used to test the hypothesis that haplodiploids have reduced levels of genetic variability. A comparison of hymenopterans with diplodiploid non-hymenopteran insects shows that hymenopterans are significantly less variable (Mann-Whitney U test, one-tailed

$p < .00003$). This is consistent with the separate findings of other workers (Snyder, 1974; Metcalf et al., 1975; Pamilo et al., 1978a, 1978b; Lester and Selander, 1979).

If haplodiploidy *per se* is at the core of the observed differences, we would expect to see the same patterns in other haplodiploids. In the only other haplodiploids studied, the phytophagus mites *Tetranychus urticae*, Helle (1965, 1968) and McEnroe (1968) found nothing to indicate reduced variability. More extensive examination of non-hymenopteran haplodiploids is crucial to any general theory of reduced variability among haplodiploids.

It is possible that variability is routinely underestimated in hymenopterans. Resolution of variation and its genetic interpretation may be poorer among hymenopterans because of their relatively heavy concentrations of pheromones, many of which are organic acids and terpenes (Blum, 1973). Other reasons for poor resolution of variation (Hung and Vinson, 1977) are not unique to Hymenoptera. Data lost due to pheromone interference would include a disproportionate number of variable loci and would thus bias mean heterozygosity to appear lower than in species with less pheromone interaction. Some workers have avoided this problem in Hymenoptera by assaying only the heads of animals (e.g. Johnson et al., 1969). This was impossible for the species studied due to the minute size of individuals (2 to 4 mm long).

To test the hypothesis that underestimation of variability in Hymenoptera is sufficient to account for the low levels of variability observed, all *H* values for Hymenoptera reported on in Table 7 were multiplied by 1.33 (This value was chosen because it represents the

greatest ratio of $\underline{H}$ to $\underline{H}^*$ found in this study and, thus, the factor which would compensate for the maximum underestimate of genetic variability possible with these data.) These transformed $\underline{H}$ values were then compared to non-transformed values for non-hymenopterans. The tendency for hymenopterans to show low levels of genetic variability is robust to this transformation of the data. Therefore, although there is the potential of having significantly lower estimates of variability due to poor resolution, the data examined show that this bias is not sufficient to account for the generally reduced levels of heterozygosity. Therefore, there must be a biological basis, beyond the physico-chemical one, for low $\underline{H}$ values.

CONCLUSIONS

This study has demonstrated the following:

1. The mean heterozygosity (H) in a total of 16 populations of three species of dolichoderine ants ranges among species from 0.035 to 0.102. The mean for all three species is 0.059. The percent polymorphic loci per population (P) ranges among species from 21% to 39% with a mean of 28%.
2. Heterozygosity values for the three species studied are consistent with those found in other Hymenoptera and are low when compared with non-hymenopteran insects.
3. Heterozygosity among advanced eusocial Hymenoptera does not differ from that among solitary species. Primitively eusocial species show lower levels of variability than do either those with more complex societies or solitary species.
4. Among ant species, there is no difference in population levels of variability between monogynous and polygynous species. However, within the three species studied here, the most polygynous, *Conomyrma bicolor* has the highest levels of genetic variability. Heterozygosities (H) are over twice those found in the less polygynous *Iridomyrmex pruinosum* and the monogynous *C. insana*.

LITERATURE CITED

Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourão, and S. Pérez-Salas, 1972. Enzyme variability in the Drosophila willistoni group. IV. Genic variation in natural populations of Drosophila willistoni. Genetics 70:113-139.

Blum, M. S., 1973. Comparative exocrinology of the Formicidae. Proc. VII Congr. IUSSI, London, pp. 23-40.

Bodenheimer, F. S., 1937. Population problems of social insects. Biol. Rev. 12:393-430.

Cockley, D. E., J. L. Gooch, and D. P. Weston, 1977. Genetic diversity in cave dwelling crickets (Ceuthophilus gracilipes). Evolution 31:313-318.

Crow, J. G. and M. Kimura, 1970. An Introduction to Population Genetics Theory. Harper & Row, Publishers, New York, 591 pp.

Crozier, R. H., 1970. On the potential for genetic variability in haplo-diploidy. Genetica 40:551-556.

Crozier, R. H., 1976. Counter-intuitive property of effective population size. Nature 262:384.

Crozier, R. H., 1977. Evolutionary genetics of the Hymenoptera. Annu. Rev. Entomol. 22:263-288.

Halliday, R. B., 1978. Genetic studies of meat ants (Iridomyrmex purpureus). Ph.D. Thesis, Dept. of Genetics, Univ. of Adelaide.

Hamilton, W. D., 1964. The genetical evolution of social behaviour. I and II. J. Theor. Biol. 7:1-52.

Helle, W., 1965. Inbreeding depression in an arrhenotokous mite. Entomol. Exp. Appl. 8:299-304.

Helle, W., 1968. Genetic variability of photoperiodic response in an arrhenotokous mite (Tetranychus urticae). Entomol. Exp. Appl. 11:101-113.

Hung, A. C. F. and S. B. Vinson, 1977. Electrophoretic techniques and genetic variability in Hymenoptera. Heredity 38:409-411.

Johnson, F. M., 1966. Rapid single fly homogenization for the investigation of Drosophila isozymes. Drosophila Information Service 41:193-194.

Johnson, F. M., H. E. Schaffer, J. E. Gillaspy, and E. S. Rockwood., 1969. Isozyme genotype-environment relationships in natural populations of the harvester ant Pogonomyrmex barbatus, from Texas. Biochem. Genet. 3: 429-450.

Krepp, S. R. and M. H. Smith., 1974. Genic heterozygosity in the 13-year cicada. Evolution 28:396-401.

Laing, C., G. R. Carmody and S. B. Peck, 1976. Population genetics and evolutionary biology of the cave beetle Ptomaphagus hirtus. Evolution 30:484-498.

Lester, L. J. and R. K. Selander, 1979. Population genetics of haplodiploid insects. Genetics 92: 1329-1345.

Levins, R., 1968. Evolution in Changing Environments. Princeton University Press, Princeton, New Jersey, 120 pp.

Lokki, J., E. Suomalainen, A. Saura, and P. Lankinen, 1975. Genetic polymorphism and evolution in parthenogenetic animals. II. Diploid and polyploid Solenobia triquetrella (Lepidoptera: Psychidae). Genetics 79:513-525.

McEnroe, W. D., 1969. Free genetic variability in the two-spotted spider mite Tetranychus urticae K. (Acarina: Tetranychidae). Massachusetts Agric. Exp. Sta., U. Massachusetts Waltham Field Sta., Exp. Bull. No. 580.

Metcalf, R. A., J. C. Marlin, and G. S. Whitt, 1975. Low levels of genetic heterozygosity in Hymenoptera. Nature 257: 792-794.

Pamilo, P., R. Rosengren, K. Vepsäläinen, S. Varvio-Aho, and B. Pisarski, 1978a. Population genetics of Formica ants. I. Patterns of enzyme gene variation. Hereditas 89:233-248.

Pamilo, P., S. Varvio-Aho, and A. Pekkarinen, 1978b. Low enzyme gene variability in Hymenoptera as a consequence of haplodiploidy. Hereditas 88:93-99.

Plateaux-Quénu, C. 1962. Biology of Halictus marginatus Brulle. J. Apic. Res. 1:41-51.

Poulik, M. D., 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180:1477-1479.

Powell, J. R. 1975. Protein variation in natural populations of animals, pp. 79-119. In Dobzhansky, Hecht, and Steere (eds.), Evolutionary Biology, v. 8. Plenum Press, New York and London.

Saura, A., O. Halkka, and J. Lokki, 1973. Enzyme gene heterozygosity in small island populations of Philaenus spumarius (L). (Homoptera). Genetica 44:459-473.

Scherba, G., 1961. Nest structure and reproduction in the mound-building ant Formica opaciventris Emery in Wyoming. J. N. Y. Entomol. Soc. 69:71-87.

Selander, R. K. and W. E. Johnson, 1972. Genetic variation among vertebrate species. Proc. XVII Int. Congr. Zool. Monaco.

Selander, R. K. and D. W. Kaufman, 1973. Genic variability and strategies of adaptation in animals. Proc. Nat. Acad. Sci. 70:1875-1877.

Sluss, T. P., E. S. Sluss, H. M. Graham, and M. DuBois, 1978. Allozyme differences between Heliothis virescens and H. zea. Ann. Entomol. Soc. Am. 71(2):191-195.

Sluss, T. P. and H. M. Graham, 1979. Allozyme variation in natural populations of Heliothis virescens. Ann. Entomol. Soc. Amer. 72:317-322.

Snyder, T. P., 1974. Lack of allozymic variability in three bee species. Evolution 28(4):687-688.

Suomalainen, E. and A. Saura, 1973. Genetic polymorphism and evolution in parthenogenetic animals. I. Polyploid Curculionidae. Genetics 74:489-508.

Sylvester, H. A., 1976. Allozyme variation in honeybees (Apis mellifera L.). Ph.D. Thesis, Univ. of California, Davis.

Trivers, R. L. and H. Hare, 1976. Haplodiploidy and the evolution of the social insects. Science 191:249-263.

Varvio-Aho, S., O. Järvinen and K. Vepsäläinen, 1978. Enzyme gene variation in three species of water striders (Gerris). Ann. Entomol. Fenn. 44:87-93.

Ward, P. S., 1978. Genetic variation, colony structure, and social behaviour in the Rhytidoponera impressa group, a species complex of ponerine ants. Ph.D. Thesis, Univ. of Sydney.

Wilson, E. O., 1963. Social modifications related to rareness in ant species. Evolution 17:249-253.

Wilson, E. O., 1966. Behaviour of social insects. Symposium of the Royal Entomological Society of London, 3:81-96.

Wilson, E. O., 1971. The Insect Societies. Harvard Univ. Press, Cambridge, Massachusetts, 548 pp.

CHAPTER 3. An electrophoretic analysis of queen number in three species of dolichoderine ants

INTRODUCTION

Ant colonies often contain more than one potentially reproductive female. In many cases, however, these apparently polygynous colonies may be functionally monogynous (Baroni-Urbani, 1968; Buschinger, 1968, 1970; Hölldobler and Wilson, 1977). The most reliable way of ascertaining whether a species or population is principally monogynous or polygynous is by examining the distribution of offspring genotypes within individual colonies. In this paper, I compare observed genotype frequencies for three species of ants with those predicted by four different mating system models to determine whether each of these species is functionally monogynous or polygynous.

The species studied were Conomyrma insana, C. bicolor, and Iridomyrmex pruinosum, all members of the subfamily Dolichoderinae. Partially excavated C. insana nests in southern California have been found to have at most one dealate female, while nests of C. bicolor and I. pruinosum have contained 3 to 7 queens (Roy R. Snelling, pers. comm.; Berkelhamer, unpubl. data). Field observations on swarming C. bicolor and laboratory colony founding data for C. bicolor and C. insana (see Chapter 5) corroborate excavation findings. Taken together, excavation, behavioral, and demographic data led me to predict that C. insana would prove to be monogynous while C. bicolor and I. pruinosum could be polygynous. These predictions are tested by genetic analysis and the fit of observed data to models of differing social structures.

THE MODELS

Most Hymenoptera, including ants are haplodiploid; males are parthenogenetic products of haploid eggs and females arise from fertilized, diploid eggs. Consequently, ant colonies with a single queen, singly inseminated may exhibit at most two genotypes at a given locus among diploid female workers, since the paternal complement will be constant. Genotype distributions within colonies of more complex parentage can also be predicted, and various mating system models can then be tested. For each of the three species studied, one polymorphic enzyme system, of straightforward genetic basis, was analyzed to assess the probability of fit to a variety of possible mating systems.

One might imagine a continuous spectrum of possible mating systems, ranging from single-queens, singly inseminated through many queens, multiply inseminated. As a first approximation, four representative models were selected for testing. These models were chosen for their simplicity and for the distinctiveness of their predictions.

The four mating system models tested were: (1) all colony offspring are the progeny of one reproductive female, singly inseminated, (2) colony offspring are the progeny of one reproductive female, doubly inseminated, (3) colony offspring are the progeny of two reproductive females, each singly inseminated, and (4) colony offspring are the progeny of three reproductive females, each singly inseminated.

Expected colony genotype distributions were obtained from average allele frequencies under the assumptions of the four models being tested. Predictions were made regarding the number of colonies expected to be monomorphic, having only homozygotes of the most common allele; the number expected to be dimorphic, comprised of heterozygotes as well as homozyogtes; and so on. General expected genotype distributions among colonies for a two allele system under the four tested mating systems are given in Table 1. Expected genotype distributions were compared with observed frequencies and analyzed using chi-square tests for goodness of fit.

Allele and genotype frequencies were obtained by starch gel electrophoresis followed by staining for individual enzymes. Techniques and data are summarized in Chapter 2.

The enzyme systems analyzed were as follows: phosphoglucose isomerase for Conomyrma insana, glutamate oxalate transaminase for C. bicolor, and phosphoglucomutase for I. pruinosum. For each species, these enzymes represent the most variable of the polymorphic systems whose genetic basis was well understood. In most cases, genetic interpretations of diploid female phenotypes were verified by examination of haploid male allozymes. For Iridomyrmex pruinosum, a second enzyme system (anodal malic dehydrogenase) was analyzed. This system also was well understood genetically, but levels of polymorphism were too low to use for the primary analysis of I. pruinosum.

The analyses have been done on ant colonies from 6 collecting sites (A, B, C, D, F, and G) in a valley near Palomar Mountain,

Table 1. General expected genotype distributions among colonies for a two-allele system under four different mating systems. S is the allele which migrates more slowly under the electrophoretic conditions studied; it occurs at a frequency p in the population. F is the faster allele occurring at a frequency q.

Possible genotypes present within a single colony	Expected proportion under various mating systems*			
	Model 1	Model 2	Model 3	Model 4
SS only	p^3	p^4	p^6	p^9
SS and SF	$2p^2q$	$4p^3q$	$6p^5q + 10p^4q^2 + 4p^3q^3$	$9p^8q + 30p^7q^2 + 44p^6q^3 + 27p^5q^4 + 6p^4q^5$
SS, SF, and FF	0	$4p^2q^2$	$4p^4q^2 + 8p^3q^3 + 4p^2q^4$	$4p^4q^2 + 8p^3q^3 + 4p^2q^4 + 2p^7q^2 + 16p^6q^3 + 50p^5q^4 + 50p^4q^5 + 16p^3q^6 + 2p^2q^7$
SF only	pq	$2p^2q^2$	$p^4q^2 + 2p^3q^3 + p^2q^4$	$p^6q^3 + 3p^5q^4 + 3p^4q^5 + p^3q^6$
SF and FF	$2pq^2$	$4pq^3$	$4p^3q^3 + 10p^2q^4 + 6pq^5$	$6p^5q^4 + 27p^4q^5 + 44p^3q^6 + 30p^2q^7 + 9pq^8$
SS and FF	0	0	$2p^3q^3$	$3p^6q^3 + 3p^3q^6$
FF only	q^3	q^4	q^6	q^9

* See text for explanation of mating system models.

San Diego Co., California. Details concerning site localities and collecting techniques are summarized in Chapter 2. For all colonies, a minimum of 8 workers were genotyped to determine the colony type. Most often 16 to 32 worker genotypes were obtained.

A similar approach has recently been used by Pamilo and Varvio-Aho (1979) to analyze the genetic structure of nests of the ant Formica sanguinea.

TESTS OF THE MODELS

The expected number of each colony type under the various models was calculated by substituting the allele frequencies into the appropriate expression from Table 1. The expected values were compared with those observed (Tables 2 through 7) and the goodness of fit was analyzed. Some models could be ruled out for each species.

An analysis of phosphoglucose isomerase (PGI) genotypes in Conomyrma insana is given in Table 2. Both polygynous models (models 3 and 4) are rejected ($p < .005$ for model 3; $p < .001$ for model 4). A single-queen, single-insemination mating system (model 1) is most likely ($.1 < p < .5$); but a single-queen, double-insemination system is possible, although much less likely ($.05 < p < .1$).

For Conomyrma bicolor, anodal glutamate oxalate transaminase (GOT-2) genotypes are analyzed in Table 3. Only a model that includes at least three queens per colony (model 4) can account for the observed colony genotype distributions ($.5 < p < .9$). All other models tested are rejected with $p < .05$.

The situation for Iridomyrmex pruinosum is less clear-cut. The data for phosphoglucomutase (PGM), taken as a whole, fit none of the models tested (Table 4). Although all the models are rejected probabilistically, models 1 and 2 are, in addition, rejected outright. There are observed colony genotypes which are impossible under either model. Of the two possible, albeit improbable, models, model 3 (with two queens per colony) provides a better fit than model 4 (with 3 queens per colony). This suggests that the fit would not

Table 2. Mating system analysis for Conomyrma insana using the PGI locus.

Colony type	Observed number	Expected number under various mating systems*			
		Model 1	Model 2	Model 3	Model 4
SS** only	24	27.20	25.44	22.00	17.69
SS and SF	8	4.08	7.66	11.22	15.33
SS, SF, and FF***	0	0.00	0.58	0.58	0.93
SF only	2	2.38	0.29	0.14	3.96
SF and FF	0	0.34	0.04	0.04	0.00
SS and FF****	0	0.00	0.00	0.02	0.02
FF only	0	0.00	0.00	0.00	0.00
χ^2		4.56	10.80	26.45	402.80
df		4	5	6	6
p		$.1 < p < .5$	$.05 < p < .1$	$<.005$	$<<.001$

* See text for explanation of models.
** See Table 1 for explanation of colony type symbols.
*** Impossible colony type under model 1.
**** Impossible colony type under models 1 and 2.

Table 3. Mating system analysis for Conomyrma bicolor using the GOT-2 locus.

Colony type	Observed number	Expected number under various mating systems*			
		Model 1	Model 2	Model 3	Model 4
SS** only	3	9.19	6.67	3.52	1.35
SS and SF	10	6.93	10.07	13.72	14.29
SS, SF, and FF***	10	0.00	3.80	3.79	7.27
SF only	0	4.77	1.90	0.95	0.19
SF and FF	1	2.61	1.43	1.63	0.66
SS and FF****	0	0.00	0.00	0.38	0.23
FF only	0	0.49	0.0056	0.01	0.0021
χ^2		∞	14.17	12.85	4.94
df		5	5	6	6
p		$<<.001$	$<.025$	$<.05$	$.5 < p < .9$

* See text for explanation of models.
** See Table 1 for explanation of colony type symbols.
*** Impossible colony type under model 1.
**** Impossible colony type under models 1 and 2.

Table 4. Mating system analysis for Iridomyrmex pruinosum using the PGM locus.

Colony type	Observed number	Expected number under various mating systems*			
		Model 1	Model 2	Model 3	Model 4
SS** only	18	12.53	9.68	5.42	2.43
SS and SF	2	8.09	12.22	16.85	18.31
SS, SF, and FF***	4	0.00	3.86	3.94	7.40
SF only	0	5.35	1.93	0.99	0.18
SF and FF	2	2.61	1.21	1.43	0.52
SS and FF****	1	0.00	0.00	0.36	0.24
FF only	0	0.42	0.10	0.01	0.00
χ^2		∞	∞	440.66	44600
df		6	6	6	6
p		<<.001	<<.001	<<.001	<<.001

* See text for explanation of models.
** See Table 1 for explanation of colony type symbols.
*** Impossible colony type under model 1.
**** Impossible colony type under models 1 and 2.

be improved by exploring additional models with still more queens per colony. When analyzed within localities (Table 5), one locality (F) fits all models tested beyond a single-queened, single insemination model. The data for locality F are best explained by a model based on three queens per colony. Although all models are rejected for locality A, the best fit is to model 3, which is based on two queens per colony. By contrast, the data for locality D fit a single-queened, single insemination model best. Although all models are rejected, the fit to model 1 is actually quite good considering sample size. The failure of the PGM data for I. pruinosum to fit any one model, combined with the varying tendencies among localities, suggests that no one paradigm applies. One possible explanation of the data is that most I. pruinosum colonies are single-queened, but multiple-queening is relatively common.

That polygyny occurs frequently in Iridomyrmex pruinosum is supported by data from extensive sampling of a second locus (anodal malic dehydrogenase, MDH-2) at one locality (B). Among ten colonies sampled, a rare MDH-2 allele occurs in three colonies. All other alleles among the ten colonies are the common one. For each of the three colonies carrying rare alleles, the heterozygote frequency is 0.06, and all are well below the expected value of 0.50 under single-queening with single-insemination. The probability of obtaining three such extreme values by chance alone is less than .01 for single-queened colonies, less than .05 for two queens per colony and greater than .05 for three or four queens per colony.

Table 5. Mating system analysis for Iridomyrmex pruinosum using the PGM locus - locality A alone.

Colony type	Observed number	Expected number under various mating systems*			
		Model 1	Model 2	Model 3	Model 4
SS** only	4	1.86	1.11	0.43	0.09
SS and SF	0	2.33	2.83	3.74	3.17
SS, SF, and FF***	2	0.00	1.81	1.80	3.60
SF only	0	1.90	0.91	0.44	0.11
SF and FF	1	1.46	1.16	1.34	0.77
SS and FF****	1	0.00	0.00	0.21	0.25
FF only	0	0.46	0.19	0.03	0.00
χ^2		∞	∞	36.93	176
df		6	6	6	6
p		<<.001	<<.001	<.005	<.001

* See text for explanation of models.
** See Table 1 for explanation of colony type symbols.
*** Impossible colony type under model 1.
**** Impossible colony type under models 1 and 2.

Table 6. Mating system analysis for Iridomyrmex pruinosum using the PGM locus - locality D alone.

Colony type	Observed number	Expected number under various mating systems*			
		Model 1	Model 2	Model 3	Model 4
SS** only	4	2.10	1.34	0.54	0.14
SS and SF	1	2.36	3.02	3.99	3.64
SS, SF, and FF***	0	0.00	1.70	1.69	3.38
SF only	0	1.84	0.85	0.42	0.10
SF and FF	1	1.33	0.95	1.12	0.59
SS and FF****	0	0.00	0.00	0.20	0.09
FF only	2	0.37	0.13	0.02	0.00
X^2		11.60	36.08	2244	5108
df		4	5	6	6
p		<.025	<.005	<<.001	<<.001

* See text for explanation of models.
** See Table 1 for explanation of colony type symbols.
*** Impossible colony type under model 1.
**** Impossible colony type under models 1 and 2.

Table 7. Mating system analysis for Iridomyrmex pruinosum using the PGM locus - locality F alone.

Colony type	Observed number	Expected number under various mating systems*			
		Model 1	Model 2	Model 3	Model 4
SS** only	0	0.79	0.50	0.20	0.05
SS and SF	1	0.88	1.13	1.49	1.37
SS, SF, and FF***	2	0.00	0.64	0.63	1.26
SF only	0	0.69	0.32	0.16	0.04
SF and FF	0	0.50	0.36	0.42	0.22
SS and FF****	0	0.00	0.00	0.07	0.03
FF only	0	0.14	0.05	0.01	0.00
χ^2		∞	4.13	4.00	0.87
df		5	5	6	6
p		<<.001	.5<p<.9	.5<p<.9	.975<p<.995

* See text for explanation of models.
** See Table 1 for explanation of colony type symbols.
*** Impossible colony type under model 1.
**** Impossible colony type under models 1 and 2.

Table 8. Goodness of fit of frequency of rare MDH-2 heterozygotes in *Iridomyrmex pruinosum* to mating system models (G-test) - locality B alone.

Genotype	Observed number	Expected proportion under various mating models*			
		Model 1	Model 3	Model 4	Model 5**
Heterozygote	3***	0.50	0.25	0.167	0.125
Homozygote	45***	0.50	0.75	0.833	0.875
G		36.75	9.00	3.86	1.71
df		1	1	1	1
p		<.01	<.05	.05 < p < .1	> .10

* See text for explanation of models 1, 3, and 4.
** Model 5 is with four queens, each singly inseminated.
*** Pooled data from three colonies with identical frequencies of the two genotypes.

The chi-square analyses of these data appear in Table 8. Therefore, based on a combination of PGM and MDH analyses, it is assumed that I. pruinosum is frequently multiple-queened in the areas studied.

DISCUSSION

The results of the analyses for multiple-queening (Table 9) suggest that the Conomyrma insana colonies studied are monogynous. The populations of C. bicolor and Iridomyrmex pruinosum studied are considered to be multiple-queened.

It should be stressed that these conclusions apply only to those southern California populations of these species analyzed in this paper. Elsewhere, Nickerson et al. (1975) have convincingly demonstrated that some northern Florida Conomyrma insana are polydomous (occupy multiple nests) and multiple-queened. This geographic variation in colony organization provides additional data supporting the growing evidence that C. insana is a complex of several species that are mostly allopatric (Roy R. Snelling, pers. comm.).

The analyses for I. pruinosum point up a difficulty with the techniques employed here. This procedure assumes that all colonies will fit a single model; whereas, in nature, there is undoubtedly variation in colony structure. Ward (1978) has shown that such variability exists within species of ponerine ants of the Rhitidoponera impressa group. It is hypothesized that Iridomyrmex also exhibits variability in social structure with some colonies monogynous and others polygynous. Only 19% of the Iridomyrmex colonies (5 colonies out of a total of 27, see Table 4) analyzed for PGM genotypes cannot be explained by model 1, while 42% of the C. bicolor (10 colonies out of a total of 24, see Table 3) analyzed were inconsistent with model 1. One interpretation of this is that the frequency of

Table 9. Summary of mating system model analyses. NO indicates that the model is rejected for the species at the locus analyzed with $p<.05$. A '+' indicates that the model is not rejected; and '++' that it is not rejected with $p>.95$.

Species		Model 1*	Model 2*	Model 3*	Model 4*
Conomyrma insana		+	+	NO	NO
(PGI)		.1<p<.5	.05<p<.1	p<.005	p<<.001
Conomyrma bicolor		NO	NO	NO	+
(GOT)		p<<.001	p<.025	p<.05	.5<p<.9
Iridomyrmex pruinosum		NO	NO	NO	NO
(PGM)		p<<.001	p<<.001	p<<.001	p<<.001
by separate localities	A	NO	NO	NO	NO
		p<<.001	p<<.001	p<.005	p<.001
	D	NO	NO	NO	NO
		p<.025	p<.005	p<<.001	p<<.001
	F	NO	+	+	++
		p<<.001	.5<p<.9	.5<p<.9	.975<p<.995

* See text for explanation of models.

multiple-queened colonies is lower in I. pruinosum than in C. bicolor. (If this is true, 19% and 42% are minimal estimates of the occurrence of polygynous colonies).

For all three species studied, the fit of the genetic data to the various models is consistent with the predictions from field and laboratory data (see Chapter 5).

CONCLUSIONS

It can be concluded from the results of this study that:

1. The *Conomyrma insana* colonies studied are probably monogynous.
2. A large number (at least 42%) of the *Conomyrma bicolor* colonies studied are functionally polygynous. Polygyny occurs in all localities examined.
3. Polygyny is also common in *Iridomyrmex pruinosum*. Its occurrence may be less widespread than in *C. bicolor*.

LITERATURE CITED

Baroni-Urbani, C., 1968. Monogyny in ant societies. Zool. Anz. 181: (3,4) 269-277.

Buschinger, A., 1968. Mono- und Polygynie bei Arten der Gattung Leptothorax Mayr (Hymenoptera Formicidae). Ins. Soc. 15: 217-226.

Buschinger, A., 1970. Zur Frage der Monogynie oder Polygynie bei Myrmecina graminicola (Latr.) (Hym., Form.). Ins. Soc. 17: 177-182.

Hölldobler, B. and E. O. Wilson, 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften 64: 8-15.

Nickerson, J. C., H. L. Cromroy, W. H. Whitcomb, and J. A. Cornell, 1975. Colony organization and queen numbers in two species of Conomyrma. Ann. Entomol. Soc. Amer. 68: 1083-1085.

Pamilo, P. and S. Varvio-Aho, 1979. Genetic structure of nests in the ant Formica sanguinea. Behav. Ecol. Sociobiol. 6: 91-98.

Ward, P. S., 1978. Genetic variation, colony structure, and social behavior in the Rhytidoponera impressa group, a species complex of ponerine ants. Ph.D. Thesis, Univ. of Sydney.

CHAPTER 4. Genetic structuring of ant populations

POLYGYNY AND KIN SELECTION

The relationship between genetic structure and queen number in the eusocial Hymenoptera is of critical significance to hypotheses for the evolution of sterile worker castes by kin selection. This paper examines the genetic structure of populations of three species of ants in the subfamily Dolichoderinae. These three species differ with respect to the number of functional queens per colony.

According to Hamilton's (1964) theory of the evolution of sociality in insects, sterile worker castes in the Hymenoptera have evolved as a result of the genetic system of these insects. Females are diploid, resulting from fertilized eggs, whereas males are the haploid products of parthenogenetic eggs. This condition results in the curious situation whereby, on the average, females share more genes with full sisters than with daughters. Based on this observation, Hamilton has argued that the evolution of sterile worker castes in the Hymenoptera has been favored because an individual female may increase her "inclusive fitness" more by raising her sisters (which share 3/4 of her genes, on average), than by raising her own daughters (which carry 1/2 of her genes).

Hamilton's argument assumes that there be only one queen per colony (monogyny) and that each queen be inseminated by only one male (or have available the sperm of one male at a time). Although these assumptions may often be met, they are by no means the rule. Multiple insemination is the common situation in honeybees (Brian, 1965), although the sperm of one male may be used up before that of another

is used. The number of times that female ants mate has yet to be determined for most species. The more obvious difficulty with Hamilton's assumptions is the widespread occurrence of multiple-queening (polygyny) among social Hymenoptera.

In most of the eusocial Hymenoptera, a colony is founded by a single queen, unaided by workers. However, colony founding by groups of females or secondary incorporation of additional reproductives into existing colonies is common in some species in each of the major eusocial hymenopteran groups (ants, bees, and wasps). Polygyny of one sort or another is an evolutionarily widespread phenomenon, not only in the Hymenoptera at large, but especially among ants. Polygyny occurs in species of at least five of the seven major subfamilies of the family Formicidae (to which all ants belong).

Many moderately polygynous species are rare parasitic or specialized species, found only in caves or bogs. Wilson (1963) has suggested that polygyny in these species may be an adaptation that increases effective population size in situations where deme size is extremely small. Some highly polygynous species (having up to hundreds of queens per colony at times) occur in four of the major families. Moreover, the absence of highly polygynous species from the more primitive subfamilies, combined with their sporadic occurrence among these four less primitive subfamilies suggests that this does not reflect a primitive life style. Unlike the species with which Wilson deals, none of the highly polygynous species is rare, or occurs in unusually small demes. In fact, Iridomyrmex humilis and Monomorium pharaonis, two such species, are both extremely widespread and

abundant pest species. Thus, it is likely that this life style must have evolved independently at least four times.

The evolution of permanently polygynous species of ants, without subsequent breakdown of the sterile caste structure, is difficult to explain by Hamilton's theory of kin selection. Its repeated, independent origin compounds the problem, eliminating simple, historical interpretations.

To point out some of the difficulties that polygyny presents to the Hamiltonian theory, I have constructed the following model. Let us examine the genetic consequences of one generation of polygyny:

(a) if co-queens are unrelated and each queen mates only once, randomly, or

(b) if co-queens are full sisters and each mates only once, randomly, or

(c) if co-queens are full sisters and each mates only once, with a full brother.

In all three cases to be considered, the general population level of inbreeding is assumed to be so low as to be ignored. (Obviously, if sisters and brothers routinely mate, as in case c, this will not be true.) The degree of relatedness of typical first generation offspring in the three cases examined is summarized in Table 1.

In case a, it should always be preferable for females to raise daughters instead of nestmates. One might occasionally expect to find associations of a few unrelated queens founding colonies, but never as many as 10. If the females are full sisters, associations of a few females should be relatively common, but one would not expect to find

Table 1. Degree of relatedness* between individuals in polygynous colonies under a variety of circumstances.

Source of queens	Source of mates**	Relationship of F_1 females to full sisters	Relationship of F_1 females to non-sister females of F_1	Number of queens	Average relationship of F_1 females	Relationship of F_1 females to future daughters
unrelated (case a)	random (case a)	75%	0%	2	37.5%	50%
				10	7.5%	50%
				100	.75%	50%
full sisters (case b)	random (case b)	75%	19%	2	47%	50%
				10	24%	50%
				100	19%	50%
full sisters (case c)	full brothers (case c)	75%	55%	2	65%	75%***
				10	57%	75%***
				100	55%	75%***

* Relatedness is expressed as the expected % of genes of one individual found in another individual.

** Single insemination of females is assumed.

*** To the extent that F_1 females are unable to distinguish males which are their sibs from those which are their cousins, this value will be diminished, as will the average relationship of future generations of females.

associations of many queens if the females practiced random mating (Table 1, case b). Only when both the co-queens and their mates (Table 1, case c) are highly related (perhaps full sibs) would a Hamiltonian argument predict that associations of many queens might evolve.

Hamilton (1972) has said, for reasons illustrated in the preceding discussion, that polygyny may prove to be the best argument against his genetical theory of the evolution of sterile worker castes in the social Hymenoptera. To overcome this objection to the theory, he proposes that polygynous social insect societies are able to evolve only in situations where the organisms have a high level of what he calls "genetic viscosity". For ants, this means that nearby colonies should be much more closely related to one another than to distant colonies. Functionally, it means that reproductive females of polygynous species should mate with males from nearby colonies and should settle, after mating, near their parent colonies.

Hamilton's (1971) "viscous" population model is one case of a group of models of population structure that produce deviations from panmixis. A variety of similar models have been proposed, including Wright's (1943, 1946) "isolation by distance" model, Wright's (1943) "island" model, and Kimura and Weiss' (1964) "stepping-stone" model. All of these models are similar in proposing alternatives to panmixis which result in local inbreeding. It is this local inbreeding that is critical to Hamilton's rejoinder to objections to the genetic theory of eusociality.

If the kinship hypothesis of the evolution of sterile castes is correct, populations of polygynous ants should be more inbred than are those of monogynous species. To test this prediction, I have compared the population genetic structure of one monogynous ant species, Conomyrma insana, with those of two closely related polygynous species, Conomyrma bicolor and Iridomyrmex pruinosum, all living in the same or contiguous habitats. Evidence for the number of queens per colony in these three colonies is given in Chapters 3 and 5.

GENETIC DATA

The genetic structure of populations of three species of ants was analyzed. Ants were collected in April, May, and June of 1977 from six localities (A, B, C, D, F, and G) in the relatively homogeneous valley to the east of Palomar Mountain, San Diego County, California. Each locality was separated from its nearest neighbor site by an average air distance of 6.3 km. The range in intersite distances was from 2 km to 23 km. Two of the species studies, Iridomyrmex pruinosum (polygynous) and Conomyrma insana (monogynous) was found at only four of the six sites.

Allele and genotype frequencies used in the analyses were obtained by starch gel electrophoresis of individual worker ants, followed by staining for specific enzymes. Techniques are summarized in Chapter 2. For the purpose of these analyses, two variable loci per species were used. For each species these represented the most variable systems studied and, thus, those in which underlying genetic patterns were most likely to be apparent. The systems studied were as follows: for C. insana, phosphoglucose isomerase (PGI) and phosphoglucomutase (PGM); for C. bicolor, PGI and anodal glutamate oxalate transaminase (GOT-2); and for I. pruinosum, PGM and isocitrate dehydrogenase (IDH).

Allele frequencies for the six loci analyzed are given in Tables 2, 3, and 4. All loci analyzed are variable within at least two of the sites studied for the species in question. It should be noted that both loci studied for Conomyrma bicolor (one of the two polygynous species) are considerably more variable than are any of the

Table 2. Allele frequencies at the PGI* and PGM loci in Conomyrma insana, a single-queened species.

Colony	PGI[1]	PGI[2]	PGI[3]	n	PGM[1]	PGM[2]	PGM[3]	n
A1	1.00	0.00	0.00	8	0.00	1.00	0.00	8
A2	1.00	0.00	0.00	8	0.00	1.00	0.00	8
A3	1.00	0.00	0.00	40	0.00	1.00	0.00	24
A4	1.00	0.00	0.00	16	--	--	--	0
A5	0.90	0.10	0.00	24	--	--	--	0
Site A**	0.98	0.02	0.00		0.00	1.00	0.00	
B1	0.50	0.50	0.00	8	0.00	1.00	0.00	16
B2	1.00	0.00	0.00	16	0.00	1.00	0.00	16
B3	0.50	0.50	0.00	11	0.00	1.00	0.00	8
B4	0.97	0.03	0.00	16	--	--	--	0
B6	1.00	0.00	0.00	16	0.00	1.00	0.00	8
B7	0.70	0.30	0.00	15	0.00	1.00	0.00	5
B8	0.78	0.22	0.00	16	0.00	1.00	0.00	5
B9	0.81	0.19	0.00	16	0.00	1.00	0.00	6
B10	1.00	0.00	0.00	16	0.00	1.00	0.00	16
B11	1.00	0.00	0.00	8	0.00	1.00	0.00	8
Site B	0.83	0.17	0.00		0.00	1.00	0.00	
C1	0.73	0.00	0.27	24	0.00	0.50	0.50	16
C2	0.76	0.00	0.24	40	0.00	0.50	0.50	8
C3	1.00	0.00	0.00	24	0.00	1.00	0.00	3
C4	1.00	0.00	0.00	8	--	--	--	0
C5	0.94	0.06	0.00	16	--	--	--	0
Site C	0.89	0.01	0.10		0.00	0.67	0.33	
D1	0.50	0.00	0.50	16	0.00	1.00	0.00	16
D2	1.00	0.00	0.00	24	0.00	1.00	0.00	24
D3	1.00	0.00	0.00	16	0.00	1.00	0.00	16
D5	1.00	0.00	0.00	16	0.00	1.00	0.00	8
D6	1.00	0.00	0.00	16	0.36	0.64	0.00	14
D7	1.00	0.00	0.00	16	0.00	1.00	0.00	8
Site D	0.92	0.00	0.08		0.06	0.94	0.00	
G1	1.00	0.00	0.00	8	0.00	1.00	0.00	8
G2	1.00	0.00	0.00	26	0.00	1.00	0.00	8
G3	1.00	0.00	0.00	8	--	--	--	0
G4	1.00	0.00	0.00	8	--	--	--	0
Site G	1.00	0.00	0.00		0.00	1.00	0.00	
Population	0.92	0.04	0.04		0.01	0.92	0.07	

* Locus and allele symbols are as explained in text, Chapter 2.
** Site and population rows list mean allele frequencies.
n Number of workers assayed.

Table 3. Allele frequencies at the GOT-2* and PGI loci in Conomyrma bicolor, a multiple-queened species.

Colony	GOT-2^1	GOT-2^2	n	PGI^1	PGI^2	n
A1	1.00	0.00	16	0.75	0.25	16
A2	0.34	0.66	32	0.65	0.35	17
A3	0.44	0.56	32	0.50	0.50	16
A4	0.48	0.52	24	0.59	0.41	16
Site A**	0.57	0.43		0.62	0.38	
B1	0.84	0.16	32	0.75	0.25	8
B2	1.00	0.00	8	0.73	0.27	22
B3	0.79	0.21	24	0.12	0.88	8
B4	0.78	0.22	16	0.56	0.44	8
B5	0.69	0.31	24	0.50	0.50	8
BC5	1.00	0.00	8	0.63	0.37	8
B6	0.78	0.22	16	0.43	0.57	23
B7	0.81	0.19	13	0.87	0.13	15
B8	0.72	0.28	16	0.86	0.14	22
Site B	0.82	0.18		0.61	0.39	
D1	0.81	0.19	16	0.81	0.19	8
D2	0.22	0.78	16	0.72	0.28	20
D3	0.91	0.09	16	0.59	0.41	16
D4	0.69	0.31	16	0.62	0.38	8
D5	0.94	0.06	16	0.34	0.66	16
D6	0.84	0.16	16	0.34	0.66	16
D7	0.88	0.12	16	0.34	0.66	8
D8	0.72	0.28	16	0.50	0.50	16
Site D	0.75	0.25		0.53	0.47	
F1	0.47	0.53	32	0.69	0.31	19
F2	0.69	0.31	31	0.33	0.67	17
F3	0.58	0.42	32	0.59	0.41	22
Site F	0.58	0.42		0.54	0.46	
Population	0.68	0.32		0.58	0.42	

* For explanation of loci and allele symbols see text, this chapter and Chapter 2.

** Site and population rows list mean allele frequencies as explained in test.

n Number of workers assayed.

Table 4. Allele frequencies at the PGM* and IDH loci in Iridomyrmex pruinosum, a multiple-queened species.

Colony	PGM[1]	PGM[2]	n	IDH[1]	IDH[2]	IDH[3]	n
A1	0.12	0.88	32	0.00	1.00	0.00	16
A2	1.00	0.00	32	0.00	1.00	0.00	8
A3	1.00	0.00	8	0.25	0.75	0.00	24
A4	0.19	0.81	23	0.14	0.86	0.00	7
A5	0.34	0.66	32	0.06	0.94	0.00	8
A6	1.00	0.00	24	0.03	0.97	0.00	16
A7	0.25	0.75	16	0.00	1.00	0.00	8
A8	1.00	0.00	24	0.00	1.00	0.00	8
Site A**	0.61	0.39		0.06	0.94	0.00	
C1	1.00	0.00	16	0.00	1.00	0.00	32
C2	1.00	0.00	24	0.00	1.00	0.00	16
C3	1.00	0.00	24	0.00	1.00	0.00	16
C4	1.00	0.00	16	0.00	1.00	0.00	8
Site C	1.00	0.00		0.00	1.00	0.00	
D1	1.00	0.00	16	0.00	1.00	0.00	16
D2	1.00	0.00	8	0.00	1.00	0.00	24
D3	1.00	0.00	16	0.00	1.00	0.00	8
D4	0.31	0.69	24	0.00	1.00	0.00	16
D5	0.77	0.23	24	0.00	0.94	0.06	8
D6	1.00	0.00	16	0.00	1.00	0.00	8
D7	0.00	1.00	8	--	--	--	0
D8	0.00	1.00	8	--	--	--	0
Site D	0.64	0.36		0.00	0.99	0.01	
F1	0.78	0.22	16	0.00	1.00	0.00	8
F2	0.40	0.60	39	0.00	1.00	0.00	24
F3	0.75	0.25	6	0.00	1.00	0.00	6
Site F	0.64	0.36		0.00	1.00	0.00	
G1	1.00	0.00	24	0.00	1.00	0.00	16
G2	1.00	0.00	16	0.00	1.00	0.00	16
G3	1.00	0.00	32	0.00	1.00	0.00	32
G4	1.00	0.00	16	0.00	0.90	0.10	24
G5	1.00	0.00	8	0.00	0.91	0.09	23
Site G	1.00	0.00		0.00	0.96	0.04	
Population	0.78	0.22		0.01	0.98	0.01	

* Locus and allele symbols are as explained in text, Chapter 2.
** Site and population rows list mean allele frequencies.
n Number of workers assayed.

loci studied in the other two species. Heterozygosity (H), a measure of genetic variation in populations, was calculated for the three species based on samples of 8 or 9 loci (see Chapter 2). Again the value for C. bicolor (H = .102) is considerably higher than that for either C. insana (monogynous, H = .035) or I. pruinosum (polygynous, H = .039).

ANALYSES OF GENETIC STRUCTURE

A variety of methods for measuring population structure have been devised (see Schaal, 1975 for review). Each of these has its own limitations and assumptions. Based on the kind of data available and the information I wished to derive from it, I chose to analyze population structure using the genetic distance measure of Nei (1972) and the F-statistics of Wright (1943, 1951, 1965).

Analysis Using Genetic Distance

Genetic distance (D) is a measure of the accumulated number of codon differences per locus between any pair of organisms. It is applicable without regard to ploidy or mating scheme (Nei, 1972). It is defined as

$$D = - \ln I,$$

where I is the normalized identity of genes between two populations, X and Y, with respect to all loci studied. I is calculated as follows. If we let x_i and y_i be the frequencies of the i^{th} alleles at a particular locus in organisms X and Y, respectively, then the probability of identity of two randomly chosen genes at that locus from population X is $j_x = \Sigma x_i^2$ and from population Y it is $j_y = \Sigma y_i^2$. The probability of identity of two randomly chosen genes, one from X and one from Y is $J_{xy} = \Sigma x_i y_i$. The normalized identity of genes between X and Y with respect to all loci is defined as

$$I = J_{xy}/(J_x J_y)^{\frac{1}{2}},$$

where J_x, J_y, and J_{xy} are the arithmetic means of j_x, j_y, and j_{xy}, respectively, over all loci (Nei, 1972).

Although it is usually desirable to calculate D over a large number of loci (Nei and Roychoudhury, 1974), in this study it proves preferable to calculate D over only the two most variable loci per species. My interest is not in the absolute value of D for each population, but, rather in relative values of D among subpopulations of 3 ant species. Although the information contributed by adding monomorphic loci to the calculations would greatly increase confidence in the value of D as a measure of the number of codon differences per locus between subpopulation on an absolute scale, in reality the number of differences is extremely low for all comparisons. Therefore, to maximize differences for analysis, monomorphic loci and loci variable within only one subpopulation were eliminated from the analyses. This modified use of D should be kept in mind when examining the data which, therefore, should not be compared with published values of D between populations of species in which D includes monomorphic loci.

Genetic distance indices were calculated at a variety of levels as follows. For each of the six sites, mean allele frequencies within the two enzyme systems studied for each species were calculated. From these site means, an overall, or population, mean was calculated (as the unweighted mean of the site means) for each allele of each system. These values are included with the allele frequencies in Tables 2-4. Allele frequencies among the workers of a colony were then compared both with the mean frequencies for the site in which the colony

occurred, and with population means, and $\underline{D}$ was calculated. In addition, the genetic distance between each site mean and the population mean was calculated. In this way, a measure of differentiation was obtained for each colony relative to both the site and the whole population and for each site relative to the whole population. These various measures of differentiation within species were then compared between species to test Hamilton's predictions about the population genetic structure of multiple-queened eusocial Hymenoptera.

By analogy with standard notation for Wright's (1965) $\underline{F}$-statistics, I will call these three hierarchical measures of genetic distance $\underline{D}_{IT}$, $\underline{D}_{IS}$, and $\underline{D}_{ST}$ where these represent, respectively, the genetic distance between individuals and the total population, individuals and the subpopulation (or site), and subpopulations and the total population. For each subpopulation and colony, the appropriate $\underline{D}$ values have been calculated and are presented in Tables 5, 6, and 7, and Figure 1.

According to Hamilton's predictions, we would expect greater $\underline{D}_{ST}$ values for the two multiple-queened species (C. bicolor and I. pruinosum) than for the single-queened species (C. insana). If the proposed inbreeding were extremely localized, we might expect similar directional differences in $\underline{D}_{IS}$ values, and consequently in $\underline{D}_{IT}$ values as well. These predictions were tested and data and results are summarized in Figure 1 and Table 8. The median values for $\underline{D}_{IT}$, $\underline{D}_{IS}$, and $\underline{D}_{ST}$ are all greater for the multiple-queened species than for the single-queened species. Of the six comparisons made, three are significant (Mann-Whitney U test, one-tailed $p < .05$). Of the three

Table 5. Genetic distances (D) between colonies, site means, and population mean for PGM and PGI in Conomyrma insana.

Colony	D_{IT}*	D_{IS}**	D_{ST}***
A1	.0022	.0001	
A2	.0022	.0001	
A3	.0022	.0001	
Site A			.0019
B1	.1313	.0678	
B2	.0022	.0128	
B3	.1313	.0678	
B6	.0022	.0128	
B7	.0391	.0094	
B8	.0191	.0013	
B9	.0138	.0002	
B10	.0022	.0128	
B11	.0022	.0128	
Site B			.0109
C1	.1549	.0410	
C2	.1584	.0343	
C3	.0022	.0558	
Site C			.0401
D1	.1328	.1150	
D2	.0022	.0029	
D3	.0022	.0029	
D5	.0022	.0029	
D6	.0669	.0587	
D7	.0022	.0029	
Site D			.0030
G1	.0022	.0000	
G2	.0022	.0000	
Site G			.0022

* D_{IT} is the genetic distance between colonies and the total population.

** D_{IS} is the genetic distance between colonies and the subpopulation.

*** D_{ST} is the genetic distance between subpopulations and the total.

Table 6. Genetic distances (D) between colonies, site means, and population mean for GOT-2 and PGI in Conomyrma bicolor.

Colony	D_{IT}*	D_{IS}**	D_{ST}***
A1	.0826	.1395	
A2	.1183	.0514	
A3	.0621	.0310	
A4	.0388	.0087	
Site A			.0133
B1	.0407	.0144	
B2	.0791	.0247	
B3	.1785	.1942	
B4	.0084	.0031	
B5	.0053	.0232	
BC5	.0686	.0179	
B6	.0272	.0285	
B7	.0734	.0477	
B8	.0639	.0565	
Site B			.0160
D1	.0525	.0622	
D2	.2199	.3083	
D3	.0382	.0197	
D4	.0019	.0105	
D5	.0923	.0505	
D6	.0672	.0353	
D7	.0761	.0402	
D8	.0066	.0015	
Site D			.0060
F1	.0548	.0333	
F2	.0558	.0524	
F3	.0095	.0024	
Site F			.0104

* D_{IT} is the genetic distance between colonies and the total population.

** D_{IS} is the genetic distance between colonies and the subpopulation.

*** D_{ST} is the genetic distance between subpopulations and the total.

Table 7. Genetic distances (D) between colonies, site means, and population mean for PGM and IDH in Iridomyrmex pruinosum.

Colony	D_{IT}*	D_{IS}**	D_{ST}***
A1	.2886	.1553	
A2	.0221	.0806	
A3	.0662	.1299	
A4	.2674	.1365	
A5	.1343	.0525	
A6	.0239	.0725	
A7	.1894	.0893	
A8	.0221	.0806	
Site A			.0180
C1	.0221	.0000	
C2	.0221	.0000	
C3	.0221	.0000	
C4	.0221	.0000	
Site C			.0221
D1	.0221	.0672	
D2	.0221	.0672	
D3	.0221	.0672	
D4	.1482	.0730	
D5	.0002	.0128	
D6	.0221	.0672	
Site D			.0119
F1	.0001	.0117	
F2	.0955	.0384	
F3	.0007	.0073	
Site F			.0121
G1	.0221	.0006	
F2	.0221	.0006	
G3	.0221	.0006	
G4	.0316	.0015	
G5	.0302	.0011	
Site G			.0248

* D_{IT} is the genetic distance between colonies and the total population.

** D_{IS} is the genetic distance between colonies and the subpopulation.

*** D_{ST} is the genetic distance between subpopulations and the total.

Figure 1. Median values of $\underline{D}_{IT}$, $\underline{D}_{IS}$, and $\underline{D}_{ST}$ for one monogynous ant species, Conomyrma insana, and two polygynous species, C. bicolor and Iridomyrmex pruinosum. See text for explanation of D calculations. $\underline{D}_{IT}$ measures genetic distance between individual colonies and the total population to which they belong. $\underline{D}_{IS}$ measures genetic distance between individual colonies and the subpopulation within which they are found. $\underline{D}_{ST}$ measures genetic distance between subpopulations and the population as a whole.

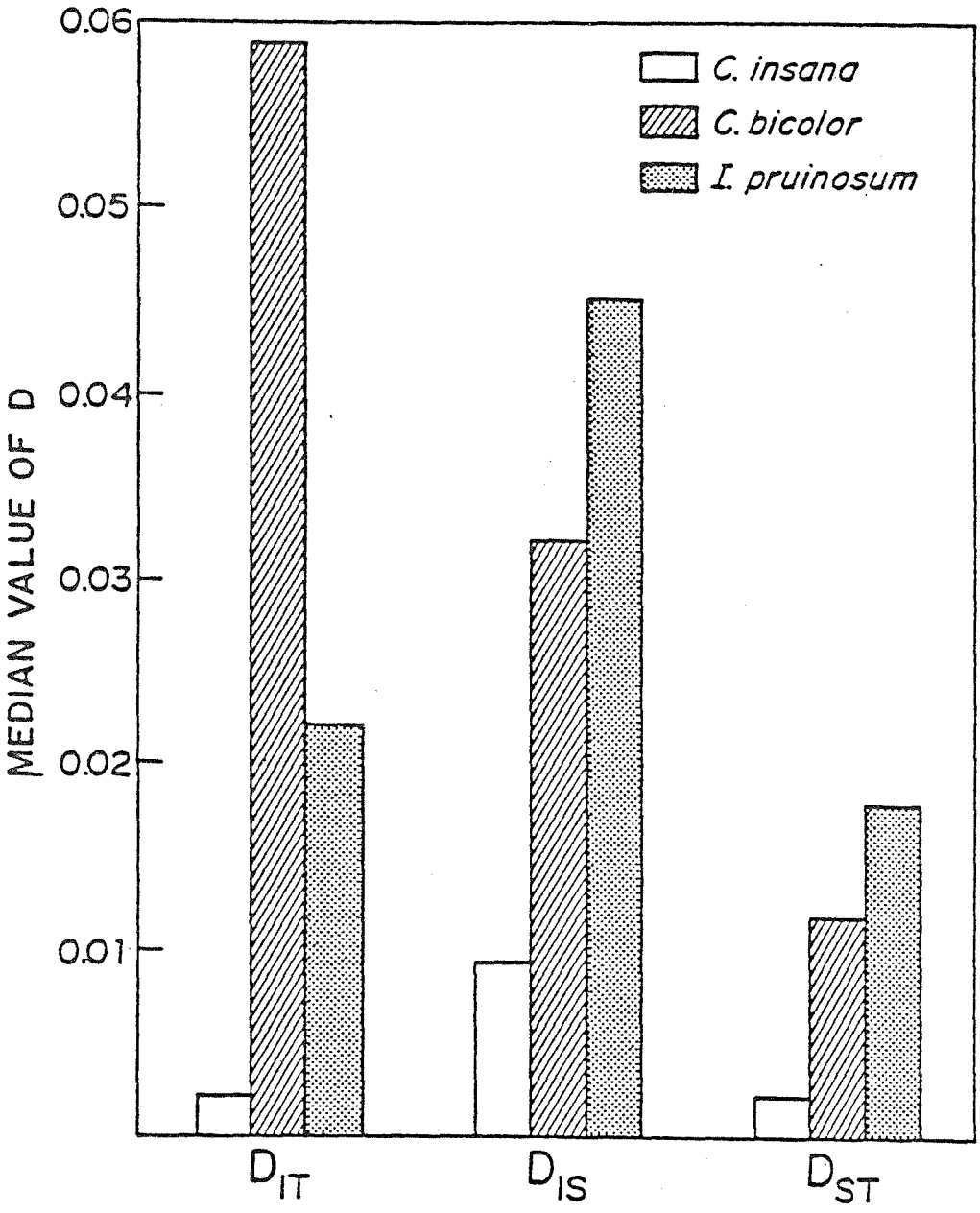

0.06
0.05
0.04
0.03
0.02
0.01
MEDIAN VALUE OF D
C. insana
C. bicolor
I. pruinosum
D_{IT}
D_{IS}
D_{ST}

Table 8. The predicted direction of difference is indicated by an inequality expression. The agreement or disagreement of the actual direction of difference between medians with that predicted if polygynous species are more inbred is given as a "+" (agrees) or "-" (disagrees) underneath the inequality expression. For the comparison between the two polygynous species (*Conomyrma bicolor* and *Iridomyrmex pruinosum*) the prediction is that there is no difference between species. Therefore, the actual direction of difference is given underneath the prediction. The significance of differences is tested by the Mann-Whitney U test and probabilities are given. For comparisons where a directional difference is predicted, probabilities are one-tailed; where no difference is predicted, probabilities are two-tailed. Significant values are indicated by an asterisk (*).

Comparison	D_{IT}‡		D_{IS}‡		D_{ST}‡	
	medians	p	medians	p	medians	p
Iridomyrmex pruinosum (IP)	IP > CI		IP > CI		IP > CI	
X						
Conomyrma insana (CI)	+	.011*	+	.090	+	.075
Conomyrma bicolor (CB)	CB > CI		CB > CI		CB > CI	
X						
Conomyrma insana (CI)	+	.005*	+	.008*	+	.206
Conomyrma bicolor (CB)	CB = IP		CB = IP		CB = IP	
X						
Iridomyrmex pruinosum (IP)	CB > IP	.122	CB < IP	.810	CB < IP	.142

‡ Genetic distance symbols are as explained in the text.

which are not significant, two have quite low probabilities ($p = .075$ and $p = .090$). When the two polygynous species are compared, there is no consistent pattern. D_{IS} and D_{ST} vary in one direction, while D_{IT} varies in opposition to them. None of the comparisons between the polygynous species is significant. Therefore, there is a trend both within subpopulations and between subpopulations for a greater degree of genetic differentiation among the polygynous ants than among the monogynous ants studied. This relationship is consistent with Hamilton's predictions.

Analysis Using F-Statistics

Population genetic structure may also be analyzed by Wright's F-statistics. These are hierarchical measures which allow partitioning of observed patterns of genetic variation. We are concerned here only with the three F-statistics (F_{IT}, F_{IS}, and F_{ST}) which are used to analyze the genetic structure of subdivided populations. F_{IT} and F_{IS} estimate the probability that two alleles at a locus within an individual are identical. F_{IT} estimates this probability with reference to allele frequencies in the total population, while F_{IS} estimates it with reference to allele frequencies in the subpopulation to which the individual belongs. In practice, they are measured by deviations in the observed number of heterozygotes from that predicted by Hardy-Weinberg as

$$F = 1 - H/2pq,$$

where F is F_{IT} or F_{IS}, H is the observed proportion of heterozygotes, and p and q are the allele frequencies in the total population or

subpopulation, respectively. F_{ST}, the measure of variation among subpopulation, is related to the variance in allele frequencies and is defined as

$$F_{ST} = \sigma_p^2/\bar{p}\bar{q}.$$

The total variation among individuals (F_{IT}), then, is composed of three components: (1) the variation within subpopulations (F_{IS}), (2) the variation among subpopulations (F_{ST}), and (3) a covariance term. Mathematically this relationship is

$$F_{IT} = F_{IS} + F_{ST} - F_{IS} \cdot F_{ST}.$$

F-statistics are calculated for one genetic locus at a time. For the analysis reported here, they were calculated for one locus per species. The loci analyzed were as follows: PGI for Conomyrma insana, GOT-2 for C. bicolor, and PGM for Iridomyrmex pruinosum. Other loci were not sufficiently variable for analysis by F-statistics. For each locus studied, all three F-statistics were calculated. F_{ST} was calculated as the variance in allele frequency among subpopulations since this was the most meaningful in terms of the hypothesis being tested. These data are summarized in Table 9.

Since F-statistics measure variation among subpopulations in terms of deviations from Hardy-Weinberg equilibrium, large positive values indicate large deviations from random mating and a high degree of differentiation. One source of such deviation and differentiation may be inbreeding. Individual colonies of Conomyrma insana, the monogynous species, show no fixation either within subpopulations or with respect to the total population at the PGI locus. In fact the negative values of F_{IS} and F_{IT} indicate an excess of heterozygotes over

Table 9. F-statistics for 1 locus in each of three species of ants.

Species	Locus	F_{IT}	F_{IS}	F_{ST}
Conomyrma insana	PGI	-.20	-.09	.08
Conomyrma bicolor	GOT-2	.16	.09	.07
Iridomyrmex pruinosum	PGM	.62	.32	.24

that predicted under panmixis. For the two polygynous species studied, F_{IT} values are positive, indicating deviation from random mating. In both cases, the fixation is accounted for fairly equally by fixation within subpopulations and fixation between subpopulations.

The degree of fixation among subpopulations, as measured by F_{ST} varies among genera studied. The two species of Conomyrma studied have the same F_{ST} values for the loci studied, while the Iridomyrmex species studied has a value three times that of either Conomyrma species. Although there are no statistical tests available for testing the probability that the F_{ST} values for single loci are different from zero these values are all relatively high when compared with values found for other species in other studies.

The heterogeneity observed among sites for I. pruinosum and C. insana was further analyzed using genetic distance measures. The fit of the data to a simple isolation by distance model (Wright, 1943) was tested. Using the two most variable loci per species, D values were calculated for all possible intersite comparisons for all three species. In addition, D was calculated for PGM alone in I. pruinosum. (This locus was shown by G-test to be significantly heterogeneous among sites.) If isolation is simply by distance, we would expect a correlation between D and absolute distance. Values of D and air distance for all possible comparisons are given in Table 10. In no case is there a significant positive correlation between genetic distance (Spearman rank correlation analysis). Obviously it is necessary to examine several additional variable loci before any absolute conclusions can be drawn.

Table 10. Analysis of correlation between genetic distance (D) and geographic distance (d) for all possible intersite comparisons for three species of ants.

Species	Locus	Sites	D	d
Conomyrma insana	PGI & PGM	A x B	.0101	6.3 km
		A x C	.0572	6.6
		A x D	.0030	5.7
		A x G	.0002	23.0
		B x C	.0803	2.0
		B x D	.0147	6.3
		B x G	.0128	20.9
		C x D	.0546	4.9
		C x G	.0558	17.0
		D x G	.0029	15.0
Spearman rank correlation coefficient r_s = -.45, p > .05				
Conomyrma bicolor	GOT-2 & PGI	A x B	.0533	6.3
		A x D	.0373	5.7
		A x F	.0063	16.0
		B x D	.0087	6.3
		B x F	.0528	14.7
		D x F	.0261	10.3
Spearman rank correlation coefficient r_s = -.32, p > .05				
Iridomyrmex pruinsoum	PGM & IDH	A x C	.0806	6.6
		A x D	.0021	5.7
		A x F	.0021	16.0
		A x G	.0856	23.0
		C x D	.0672	4.9
		C x F	.0675	12.7
		C x G	.0006	17.0
		D x F	.0000	10.3
		D x G	.0719	15.0
		F x G	.0726	7.0
Spearman rank correlation coefficient r_s = .06, p > .05				

However, overall, the trends in the F-statistics are consistent with those found in the genetic distance analysis. There is an indication that the multiple-queened species may be more inbred, at least on a local scale than is the single-queened species studied. In addition, by the F-statistic analysis we might conclude that the effects of non-random mating or dispersal are much more extensive in Iridomyrmex at the global level than for either of the Conomyrma species.

Genetic Line Model

Analysis of allele frequency data both by modified genetic distance measures and by F-statistics suggests that, on a very local level, populations of two polygynous ant species may be more inbred than are those of a closely related monogynous species. In addition, the effects of this inbreeding and (or) limited dispersal are seen on a larger scale in one of the polygynous species, in which subpopulations are highly distinct from nearby conspecific subpopulations.

These analyses were performed on relatively few loci, and the total level of variability is not the same in all three species. One explanation for the observed patterns of variation might be that the patterning of genetic variation is correlated with its absolute level. This does not appear to be the case. C. insana (monogynous) and I. pruinosum (polygynous) have very similar levels of variability (as measured by H) and, thus, might be expected to have similar genetic structures. However, I. pruinosum has a genetic structure

more similar to that of C. bicolor, the other polygynous species, which has three times the genetic variability.

Even in comparing the two loci per species studied, there is no correlation between genetic variation and degree of differentiation as measured by D_{ST}. If we use the frequency of the uncommon alleles at a locus, averaged over the two loci as an indication of variability, the ranking, from least to most genetically variable, is: C. insana, I. pruinosum, then C. bicolor. D_{ST} values order the species from low to high: C. insana, C. bicolor, then I. pruinosum. Once again, there is no correlation with overall level of genetic variation.

In both polygynous species, D_{IS} and F_{IS} values, which estimate differentiation within subpopulations, are relatively high. Both the D_{IS} and the F_{IS} values suggest that there is more deviation from random mating within subpopulations of polygynous species than within monogynous species. Schaal (1975) found high F_{IS} values within subpopulations of a composite, Liatris cylindracea. Her interpretation was that the breeding neighborhood was, in fact, much smaller than the presumed subpopulations. Subpopulations themselves were subdivided into relatively distinct demes. Perhaps this is the case in the two polygynous species of ants studied also. At least, relative to the monogynous species, these species may breed much more locally. If mating flights are restricted in C. bicolor and I. pruinosum and colony reproduction is often by fission (see Wilson, 1971), the patterns observed within subpopulations would not be surprising. Polygyny, probable inbreeding, and reproduction by fission are often correlated and are well documented for a related species, Iridomyrmex

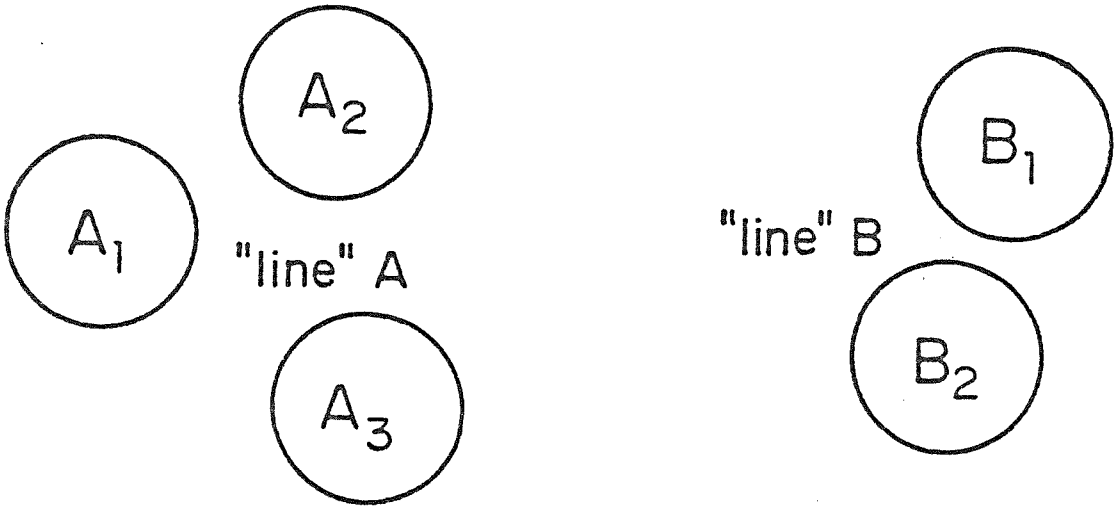
humilis, the Argentine ant (Markin, 1970). Such a reproductive pattern might result in several genetic "lines" in an area. Within a line, there would be relative homogeneity, while between lines there might be considerable distinctness. A visual representation of this genetic line model is given in Figure 2. Field data concerning colony founding in the species studied are scanty and do not allow for direct testing of these predictions.

It is possible, however, to test this model of population genetic structure by graphically analyzing the distribution of gene frequencies within sites (Figures 3-5). These graphs provide a way of visualizing genetic distances among colonies within sites. For Conomyrma insana, the monogynous species, the distribution of colonies within sites C and D fits the predictions of the genetic line model; site B may fit also (Figure 3). This fit is perplexing because the model does not apply to monogynous species. The fit is also good for Iridomyrmex pruinosum, one of the polygynous species (Figure 4). Sites A, D, and F have colonies distributed as the model predicts. Site G has too little variation to conclude much, but the trend is similar to that at other sites. Conomyrma bicolor, the second polygynous species shows the poorest fit (Figure 5). In the two sites where there is a slight clustering of genetic profiles, site A and site D, the clustering is much looser than is found in either C. insana or Iridomyrmex pruinosum. Therefore, Conomyrma bicolor subpopulations are not more viscous than are those of the monogynous congener, C. insana, whose populations are somewhat viscous for unknown reasons. I. pruinosum has viscous

populations, possibly due to locally restricted mating and (or) reproduction by colony fission.

Figure 2. Genetic line model. This figure illustrates the possible genetic structure of a polygynous ant species where reproduction is frequently by colony fission. Colonies within "lines" are genetically similar, but need not be immediately adjacent spatially. Distance between colonies, therefore, represents genetic distance.

GENETIC LINE MODEL



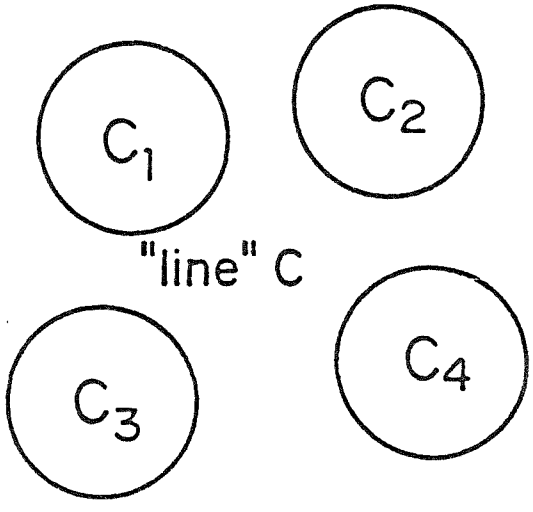
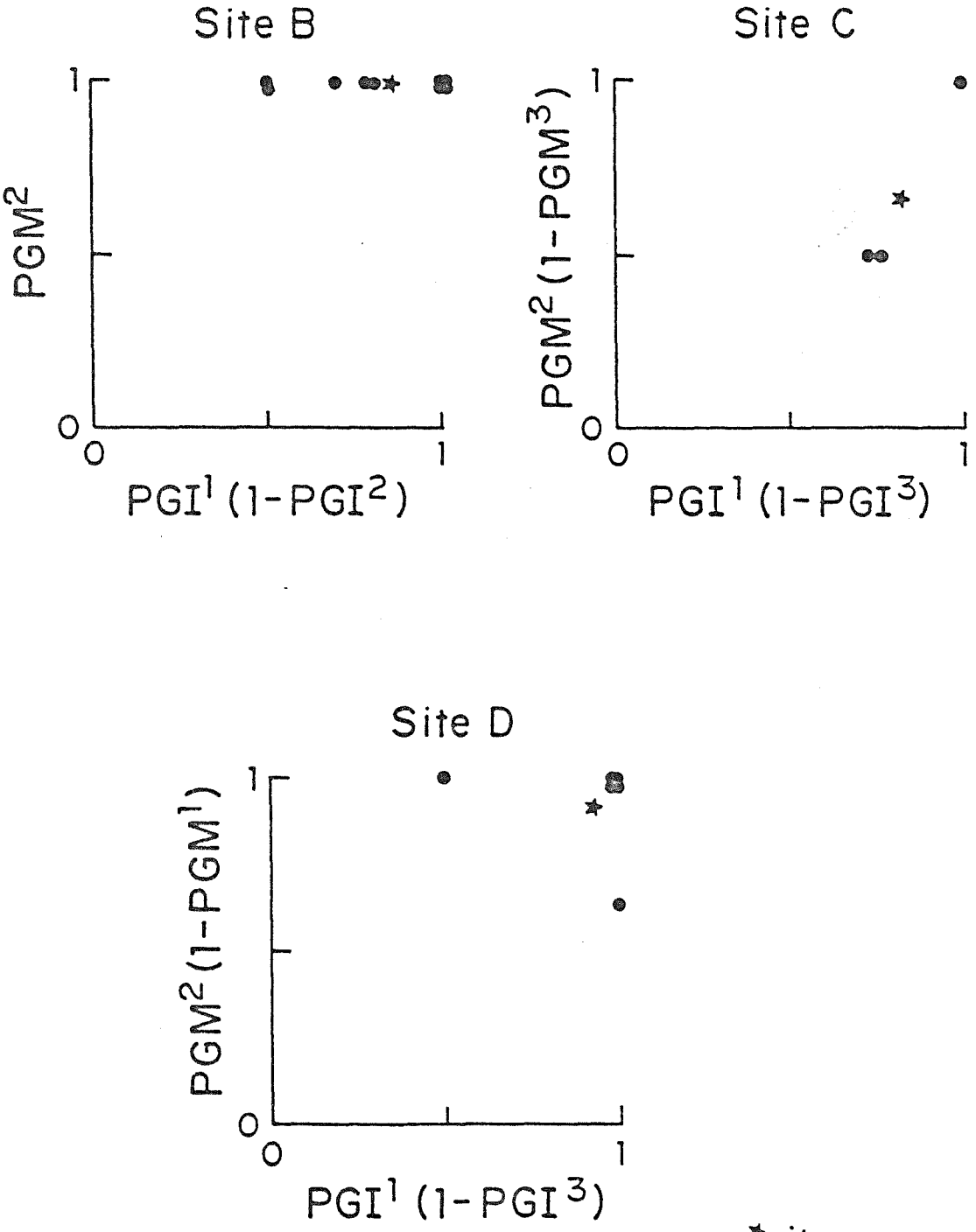


Figure 3. Within site genetic profiles for Conomyrma insana. Each colony is represented by a point in two-dimensional space which represents its allele frequencies at the two variable loci studied. All colonies within a site are plotted on the same axes to look for clustering or "genetic viscosity". Mean values for all colonies plotted at each site are indicated by an asterisk. Sites C and D appear to be genetically viscous.

Conomyrma insana



*site mean

Figure 4. Within site genetic profiles for Iridomyrmex pruinosum. Each colony is represented by a point in two-dimensional space which represents its allele frequencies at the two variable loci studied. All colonies within a site are plotted on the same axes to look for clustering or "genetic viscosity" and possible fit to the genetic "line" model (see text). Mean values for all colonies plotted at each site are indicated by an asterisk. Sites A, D, and F appear to fit the model. The trend at site G is similar, but variation is insufficient to provide conclusive evidence.

Iridomyrmex pruinosum

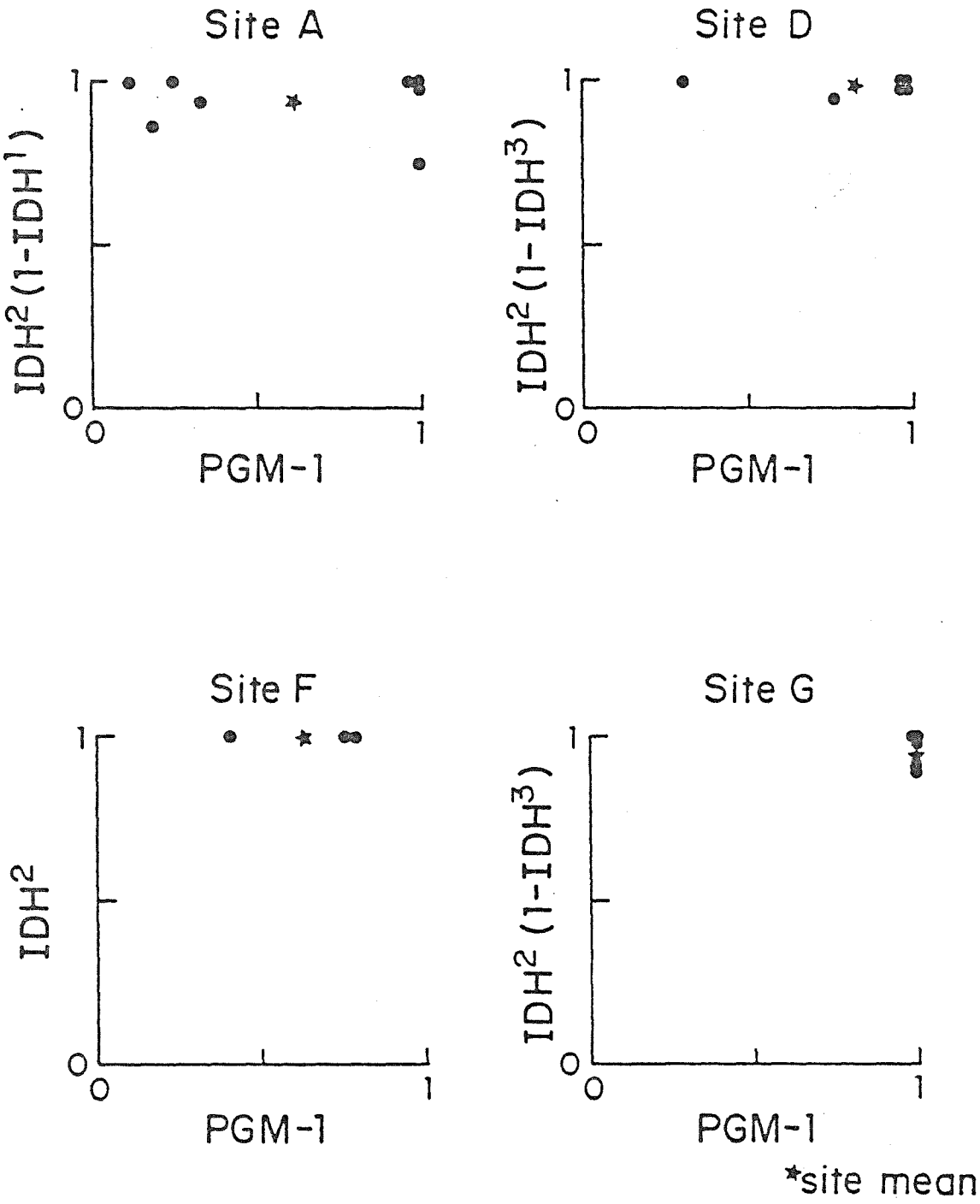
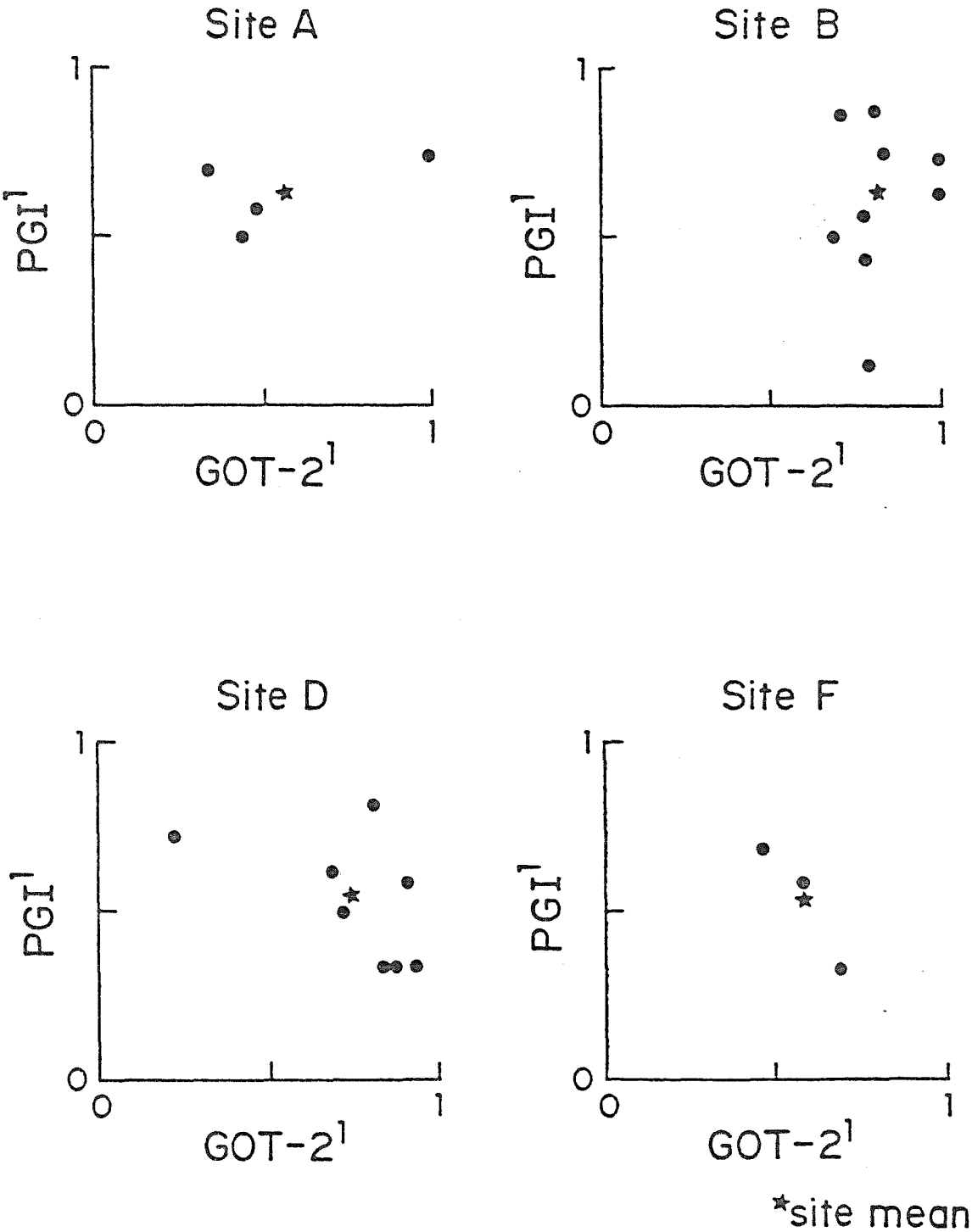


Figure 5. Within site genetic profiles for Conomyrma bicolor. Each colony is represented by a point in two-dimensional space which represents its allele frequencies at the two variable loci studied. All colonies within a site are plotted on the same axes to look for clustering or "genetic viscosity" and possible fit to the genetic "line" model (see text). Mean values for all colonies plotted at each site are indicated by an asterisk. None of the sites appears genetically viscous. At sites B and F, allele frequencies are evenly dispersed among colonies. At sites A and D, the same is true except there is one outlying colony which is quite different from the rest.

Conomyrma bicolor



DISCUSSION

How well, then, do the observed patterns of variation fit with Hamilton's predictions based on the kin selection model for the evolution of eusociality? It has already been observed that F_{ST} values are much higher in one of the polygynous species than in either the monogynous species or its polygynous congener. However, all the species studied show F_{ST} values as high as or higher than those found in other animals known to live in subdivided populations.

Population structure of American Indian tribes has been analyzed more thoroughly than that of any other group. Among both Papago and Yanomama Indians, the population is subdivided into villages that are geographically separated and among which reproduction is limited (Workman and Niswander, 1970; Arends et al., 1967). At twelve loci studied in the Papago, F_{ST} values range from .0006 to .0561 with a mean value of .0240 (Workman and Niswander, 1970). At eleven loci, the Yanomama have F_{ST} values ranging from .0329 to .0955 with a mean of .0633 (Neel and Ward, 1972). Values for all three species of ants studied are higher than the mean value for either Papago or Yanomama Indians. The values obtained for the two Conomyrma species studied are similar to those found among 8 loci in marmots (Schwartz and Armitage, 1980) for which F_{ST} ranged from .04 to .13 with a mean of .07. The value of F_{ST} found for PGM in Iridomyrmex is extremely high in comparison with values from other animal populations studied. It is also higher than F_{ST} values at 15 loci in the outbreeding composite plant Liatris cylindracea, which ranged from .0091 to .2240 with a mean

of .0687 (Schaal, 1975), but lower than F_{ST} values found at 5 loci in the facultative selfing plant, Phlox cuspidata, which ranged from .31 to .78 with a mean of .49 (Levin, 1975).

Relatively high levels of differentiation among subpopulations may be caused by a variety of factors that fall into two categories: selection, on the one hand, and nonrandom mating and drift on the other hand. From the data available, it is impossible to eliminate the effects of selection. However, nonrandom mating and drift should be perceptible at the local level as well as at the population level.

At the local level, we have already seen that the two polygynous species have greater differentiation among colonies within sites (D_{IS}) and greater deviation from expectations of random mating (F_{ST}) than does the monogynous species. Workman (1969) discusses many factors which contribute to positive F values (indicating fewer heterozygotes than predicted by Hardy-Weinberg). These factors include inbreeding, selection, mutation, gene flow, and random genetic drift. Spatial analysis of intercolony genetic distances within sites (Figures 3-5) suggests that a form of inbreeding which results from colony reproduction by budding may be a major factor for Iridomyrmex pruinosum. The positive F_{IS} value found for C. bicolor appears to have some other root, perhaps some other form of inbreeding or selection against heterozygotes.

Negative F_{IS} values indicate an excess of heterozygotes as compared with the number expected when mating is random. According to Workman (1969) this will occur whenever the fitness of the heterozygote is greater than the geometric mean of the fitnesses of

the two homozygotes. It will also occur when negative assortative mating is common. In ant species this might be accomplished by asynchronous release of male and female reproductives by a given colony or by production of only one sex of reproductives per colony. Each of these tendencies have been found in some ant species (Talbot and Kennedy, 1940; Talbot, 1945; Scherba, 1961).

Taken together, then, the analyses performed suggest the following genetic structures for the species studied. Iridomyrmex pruinosum, a multiple-queened species, has a highly subdivided population structure comprised of genetically viscous subpopulations. Viscosity within sites may be the result of colony reproduction by budding. Populations of the two Conomyrma species are subdivided, but to a much lesser extent. C. insana, a single-queened species, has random or negative assortative mating within subpopulations. A second multiple-queened species, C. bicolor, may be locally inbred (based on positive values of F_{ST}) but, if so, fails to show the expected resultant genetic viscosity. An alternative explanation of the data is that the positive F_{IS} values are a consequence of lower viability of heterozygotes at the locus studied. In sum, one of the two multiple-queened species studied behaves, genetically, as Hamilton would predict, while the other does not. Of course, in all cases, the role of selection may be more important than has been assumed in theorizing on genetic structure.

It is interesting to note that Iridomyrmex pruinosum behaves ecologically as we might expect a budding species to behave (see Chapter 6). It colonizes a chaparral habitat opened by fire very

quickly, too rapidly for colony founding to be claustral. In spite of its multiple queens, Conomyrma bicolor does not colonize the same area rapidly, although it appears to be tolerant of the conditions in the area and a source population is present. Therefore, budding may be much less common in C. bicolor than in I. pruinosum. If budding is more common in I. pruinosum, this would explain the finding of Chapter 3 that I. pruinosum colonies are probably less frequently multiple-queened than are C. bicolor colonies. Budding would serve to reduce the number of queens per colony.

It is important to point out that the greater differentiation of populations and genetic viscosity within them, in Iridomyrmex pruinosum, is consistent with Hamilton' predictions under the haplodiploidy based kin selection hypothesis of the evolution of eusociality. However, it neither provides strong evidence for the hypothesis, nor does it disallow alternative hypotheses.

It is entirely possible that kin selection has played a role in the evolution of eusociality. But need this kin selection have operated on unequal coefficients of relatedness resulting from haplodiploidy? Trivers and Hare (1976) first pointed out that although females are more closely related to sisters (3/4) than to daughters (1/2), they are less closely related to brothers (1/4) than to sons (1/2). And, therefore, when sex ratio is balanced (1:1), the advantage of raising sisters is exactly cancelled by the disadvantage of raising brothers. They predict that if haplodiploidy is the basis for kin selection of eusociality, then sex ratios among eusocial species should be female biased. In particular, Trivers and Hare

predict a 3:1 ratio of females to males. This prediction has also been made by Charnov (1979) and Benford (1979) based on models which use quite different approaches.

Although Trivers and Hare present data to substantiate their predictions, their methodology has been called into question by Alexander and Sherman (1977). The latter stress that most of the ant data presented by Trivers and Hare actually show a ratio of investment in the sexes that is greater than the predicted 3:1. They propose that an alternative hypothesis, local mate competition (Hamilton, 1967), better explains the magnitude and variation in sex ratios which Trivers and Hare find. R.K. Colwell and D.S. Wilson (pers. comm.) have proposed yet another hypothesis which might explain the Trivers and Hare data. Their model, explored by extensive computer simulation, shows that female biased sex ratios are expected in certain situations as a result of group selection.

A recent study on Polistes wasps examined sex ratios in an outbreeding population where local mate competition would be expected to be very low or absent (Noonan, 1978). A 1:1 sex ratio was found, suggesting that in Polistes fuscatus, haplodiploidy does not result in the expected skew whereby females are more closely related to sibs than to offspring. Instead, because of the 1:1 sex ratio, females are related to an average sibling at the same level as to an an average offspring. Therefore, Noonan concludes that haplodiploidy cannot be the basis for eusociality in this species. These data do not, however, eliminate the possibility that kin selection, in a broader sense, (that is, independent of haplodiploidy effects) has played a role in

the evolution of eusociality. This more general role of kin selection in the evolution of eusociality was pointed out and discussed by West Eberhard (1975).

I have suggested elsewhere (Chapter 2) that inbreeding may ultimately be the force behind the independent evolution among some hymenopteran species of both haplodiploidy and eusociality. Hamilton (1964, 1967) has also suggested that inbreeding is involved in both of these. However, his theory for the evolution of eusociality always includes an additional interaction of eusociality and haplodiploidy, so that eusociality is favored in the Hymenoptera particularly because of haplodiploidy. Alexander and Sherman (1977) have also alluded to the evolutionary independence of haplodiploidy and eusociality in the Hymenoptera and they suggest that local mate competition, a possible consequence of inbreeding, may have been the selective force behind both of these evolutionary patters. Selection to avoid local mate competition may occur at the level of individual females or at the level of the kin group. Haplodipoidy might also be selected for by group selection for skewed sex-ratios (R.K. Colwell, pers. comm.). Both the group selection and individual selection models suggest that being able to control the sex ratio of a brood would be to the advantage of the laying female or the group to which she belongs. Haplodiploidy is a mechanism by which such control can be accomplished.

Two major alternatives to haplodiploidy explanations of eusociality have been presented. These are the "mutualism" and "parental manipulation" hypotheses. Michener (1958) suggested that the ancestors of eusocial organisms may have been "pseudo-social" groups of females

of the same generation, which may or may not have been related, are hypothesized to have associated in communal nests for their mutual benefit. Perhaps they may have shared nest guarding or foraging tasks and, in so doing, have increased the probability of survival of all associated females. Michener's mutualism hypothesis has been criticized on two grounds. Firstly, there are no eusocial species in taxonomic lineages with semisocial species. Secondly, the mutualism hypothesis offers no explanation of how the transition from semi-social to eusocial might be made. What, evolutionarily, would prevent the sterile females from reproducing? Group selection is an obvious possibility, but has as yet, been avoided by most authors.

Alexander (1974) proposed a second alternate explanation for the evolution of eusociality, based on "parental manipulation" of offspring. He argues that selection may have favored reproductive females that are able to control the reproductive fate of their offspring and use some to help rear others. He makes an analogy between workers of eusocial Hymenoptera and the trophic eggs which are often laid by queens and fed to larvae. West Eberhard (1975) discusses both the mutualism and parental manipulation hypotheses in some detail. She concludes that kin selection, mutualism, and parental manipulation are not mutually exclusive factors; by contrast, they may all have contributed to the evolution of eusociality in insects.

I think it is necessary to add group selection to West Eberhard's list of factors contributing to the evolution of eusociality. Early models of group selection (Eshel, 1972; Boorman and Levitt, 1973; see also Wade, 1978 for review) concluded that the conditions under which

group selection might operate in opposition to individual selection are extremely restrictive. More recent models (Wilson, 1975, 1979; Gadgil, 1975; see Wade, 1978) have indicated that the conditions need not be as stringent as was once thought. These latter models are what Wade calls "intrademic" models.

Intrademic models of group selection are based on considerations of subdivided populations. Population members are distributed into isolated neighborhoods or "trait groups" (Wilson, 1979) for part of their life cycle (or for several generations) and are mixed together with members of other trait groups during another phase of their life cycle (or in response to some external cue). The genotypic composition of a trait group determines the relative viability and productivity of the group. Given underlying genetic variation among groups, some groups contribute more to the next generation than do others. With only random (binomial) variation among trait groups, group advantageous traits that are individually neutral will be selected for (Wilson, 1979). With somewhat more variation, individually disadvantageous traits may be selected for when advantageous to the trait group. Individuals that are equally closely related to sibs and to offspring (as is the case in diplodiploids, including termites, and female haplodiploids with 1:1 sex ratios) might be selected to rear sibs if that behavior increased the productivity of the group to which they belonged. Therefore, it is not necessary to invoke relatedness skewed toward sibs versus offspring to select for sib-rearing by group selection. It is only necessary that sib-rearing be <u>selectively neutral</u> in relation to rearing offspring. Since full sibs share the

same proportion of their genome as do parents and offspring (in diplodiploids and females of sex ratio balanced haplodiploids), then sib-rearing is selectively neutral, if there are no generational differences in reproductive value. This is a very important point which has been emphasized as a general characteristic of the trait group selection model by Wilson (1979) but which has not previously been pointed out in the eusociality arena.

For trait group selection to operate, there must be an underlying variance of genetic composition among trait groups. The idea of genetically substructured populations is replacing the classical population genetic view of populations as both infinite and panmictic. Recent studies on a variety of organisms have indicated that genetic substructuring is often found in natural populations. Organisms found to exhibit local heterogeneity include composite plants (Schaal, 1975), microtine rodents (Bowen, 1978), freshwater amphipods (Gooch and Hetrick, 1979), land snails (Selander and Kaufman, 1975; Selander and Hudson, 1976), migratory butterflies (Eanes and Koehn, 1978) and ants (this study). It remains to be seen how much of this variation is a reflection of local adaptive response. However, it is possible that a good deal of the variation is a result strictly of population substructuring, consequent local mating within small subpopulations, and subsequent random genetic drift among subpopulations.

Within the ants studied, genetic differentiation among subpopulations (F_{ST}) was found to be most pronounced in one of the polygynous species, Iridomyrmex pruinosum. In the second polygynous species, Conomyrma bicolor, variation among subpopulations was not

greater than for its monogynous congener, C. insana, but variation within subpopulations (D_{IS}) was. Therefore, trait group variation in the monogynous species is the lowest and may be assumed to be little more than random. With only random variation, selectively neutral behaviors (for example rearing of full sibs of either sex by females) are the most "altruistic" for which group selection can select. If species show more than random variation among neighborhoods, as does I. pruinosum (and, possibly, C. bicolor), then trait group selection can select for behaviors which are individually disadvantageous but group beneficial. Rearing nest mates that carry a smaller proportion of one's genes than do one's offspring, in preference to rearing one's own offspring, is an example of such a behavior. In a polygynous species, this will be the case, since not all nestmates are full sibs (Table 1). Therefore, if polygyny is a group advantageous trait, it may arise by natural selection and be maintained only in species with highly substructured populations, that is, those with genetically viscous populations.

The predictions of the group selection model are identical with those of the haplodiploidy based kin selection model. Just as with kin selection, mutualism, and parental manipulation, the hypotheses are not mutually exclusive. The various proposed selective forces may all be operating in concert, sequentially, or in any combination. For example, structured demes are also expected, by group selection, to have female biased sex ratios (R. K. Colwell and D. S. Wilson, pers. comm.). In this situation kin selection and group selection would serve to reinforce each other. As opposed to the haplodiploidy

based kin selection hypothesis, however, the group selection model can be applied to the evolution of eusociality in termites as well, and is not subject to refutation based on frequent observation of polygyny and (or) multiple insemination. Instead, it predicts that altruism should be directly correlated with $\underline{F}_{ST}$, where subpopulations represent trait groups. The group selection model is also less restrictive than the haplodiploidy based kin selection model because it operates, in principle, in the absence of female biased sex ratios, although it may produce them.

CONCLUSIONS

The analyses in this study lead to the following conclusions:

1. Both a modified form of Nei's genetic distance measure (D) and Wright's F-statistics show a trend towards greater genetic differentiation among colonies of ants within sites for the two polygynous species studied than for the monogynous species studied.
2. Graphical analses of genetic profiles within sites suggests that the relatively high differentiation found is probably due to different causes in the two species. In Iridomyrmex pruinosum, genetic structure is viscous within sites, perhaps as a result of colony reproduction by budding. In the case of Conomyrma bicolor, sites are not particularly viscous. Selection may be involved in the high F_{IS} values observed.
3. Variation among subpopulations (F_{ST}) is positive for all species studied at a level similar to that found in other animal species known to have subdivided populations. Values for one of the polygynous species, I. pruinosum, are especially high.
4. The results for the Iridomyrmex species studied are consistent with the predictions of W.D. Hamilton's haplodiploidy based kin selection model for the evolution of eusociality in the Hymenoptera. When compared with the monogynous species, Conomyrma insana, mating in the polygynous I. pruinosum appears more local and less random. The genetic structure of a second polygynous species, C. bicolor, does not fit Hamilton's predictions.

5. In this study, there is no correlation between total level of genetic variation in a species and the degree of genetic differentiation between subpopulations.
6. The distribution of genetic distances between subpopulations, within species, does not follow a simple "isolation by distance" model.
7. Although the genetic structure of I. pruinosum is as predicted for a polygynous species by the haplodiploidy kin selection model, it is also predicted by an alternate model based on group selection. The alternate model is based on D.S. Wilson's (1979) trait group selection model. The predictions of the group selection model concerning the genetic structure of polygynous species are identical with those of the kin selection model. However, the group selection model is more general because it neither requires female biased sex ratios among haplodiploids nor is it restricted to haplodiploid species.

LITERATURE CITED

Alexander, R. D., 1974. The evolution of social behavior. Ann. Rev. Ecol. Syst. 5:325-383.

Alexander, R. D. and P. W. Sherman, 1977. Local mate competition and parental investment in social insects. Science 196:494-500.

Arends, T., G. Brewer, N. Chagnon, M. L. Gallango, H. Gershowitz, M. Layrisse, J. Neel, D. Shreffler, R. Tashian, and L. Weitkamp, 1967. Intratribal genetic differentiation among the Yanomama Indians of southern Venezuela. Proc. Nat. Acad. Sci. 57:1252-1259.

Benford, F. A., 1979. Fisher's theory of the sex ratio applied to the social Hymenoptera. J. Theor. Biol. 72:701-728.

Boorman, S. A. and P. R. Levitt, 1973. Group selection on the boundary of a stable population. Theor. Popul. Biol. 4:85-128.

Bowen, B. S., 1978. Spatial and temporal patterns of genetic variation in a local population of the California vole, Microtus californicus (Rodentia: Cricetidae). Ph.D. Thesis, University of California, Berkeley.

Brian, M. V., 1965. Social Insect Populations. Academic Press, London, 135 pp.

Charnov, E. L., 1978. Sex ratio selection in eusocial Hymenoptera. Am. Nat. 112:317-326.

Eanes, W. F. and R. K. Koehn, 1978. An analysis of genetic structure in the monarch butterfly, Danaus plexippus L. Evolution 32:784-797.

Eshel, I., 1972. On the neighbor effect and the evolution of altruistic traits. Theor. Popul. Biol. 3:258-277.

Gadgil, M., 1975. Evolution of social behaviors through interpopulation selection. Proc. Nat. Acad. Sci. 72:1199-1201.

Gooch, J.L. and S. W. Hetrick, 1979. The relation of genetic structure to environmental structure: Gammarus minus in a karst area. Evolution 33:192-206.

Hamilton, W.D., 1964. The genetical evolution of social behaviour. I and II. J. Theor. Biol. 7:1-52.

Hamilton, W. D., 1967. Extraordinary sex ratios. Science 156:477-488.

Hamilton, W. D., 1971. Selection of selfish and altruistic behavior in some extreme models. In J. F. Eisenberg and W. S. Dillon, eds., Man and beast: comparative social behavior. Third Intern. Symp. Smithsonian Inst., pp. 59-91.

Hamilton, W. D., 1972. Altruism and related phenomena, mainly in social insects. Ann. Rev. Ecol. and Syst. 3:193-232.

Kimura, M. and G. H. Weiss, 1964. The stepping stone model of population structure and the decrease of the genetic correlation with distance. Genetics 49:561-576.

Levin, D. A., 1975. Interspecific hybridization, heterozygosity and gene exchange in Phlox. Evolution 29:37-51.

Markin, G. P., 1970. The seasonal life cycle of the Argentine ant, Iridomyrmex humilis (Hymenoptera: Formicidae), in southern California. Ann. Entomol. Soc. Amer. 63:1238-1242.

Michener, C. D., 1958. The evolution of social behavior in bees. Proc. Tenth Intern. Congr. Entomol., Montreal, 2:441-447.

Neel, J. V. and R. H. Ward, 1972. The genetic structure of a tribal population, the Yanomama Indians. VI. Analysis by F-statistics (including a comparison with the Makiritare and Xavante). Genetics 72:639-666.

Nei, M., 1972. Genetic distance between populations. Am. Nat. 106: 283-292.

Nei, M. and A. K. Roychoudhury, 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76:379-390.

Noonan, K. M., 1978. Sex ratio of parental investment in colonies of the social wasp Polistes fuscatus. Science 199:1354-1356.

Schaal, B. A., 1975. Population structure and local differentiation in Liatris cylindracea. Am. Nat. 109:511-528.

Scherba, G., 1961. Nest structure and reproduction in the mound-building ant Formica opaciventris Emery in Wyoming. J. N. Y. Entomol. Soc. 49:71-87.

Schwartz, O. A. and K. B. Armitage, 1980. Genetic variation in social mammals: the marmot model. Science 207:665-668.

Selander, R. K. and R. O. Hudson, 1976. Animal population structure under close inbreeding: the land snail Rumina in southern France. Am. Nat. 110:695-718.

Selander, R. K. and D. W. Kaufman, 1975. Genetic structure of populations of the brown snail (Helix aspersa). I. Microgeographic variation. Evolution 29:385-401.

Talbot, M., 1945. A comparison of flights of four species of ants. Am. Midl. Nat. 34:504-510.

Talbot, M. and C. H. Kennedy, 1940. The slave-making ant, Formica sanguinea subintegra Emery, its raids, nuptual flights and nest structure. Ann. Entomol. Soc. Amer. 33:560-577.

Trivers, R. L. and H. Hare, 1976. Haplodiploidy and the evolution of the social insects. Science 196:494-500.

Wade, M. J., 1978. A critical review of the models of group selection. Quart. Rev. Biol. 53:101-114.

West Eberhard, M. J., 1975. The evolution of social behavior by kin selection. Quart. Rev. Biol. 50:1-33.

Wilson, D. S., 1975. A theory of group selection. Proc. Nat. Acad. Sci. 72:143-146.

Wilson, D. S., 1979. The Natural Selection of Populations and Communities. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California, 186 pp.

Wilson, E. O., 1963. Social modifications related to rareness in ant species. Evolution 17:249-253.

Wilson, E. O., 1971. The Insect Societies. Belknap Press, Harvard Univ. Press, Cambridge, 548 pp.

Workman, P. L., 1969. The analysis of simple genetic polymorphisms. Human Biol. 41:97-114.

Workman, P. L. and J. D. Niswander, 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Hum. Gen. 22:24-49.

Wright, S., 1943. Isolation by distance. Genetics 28:114-138.

Wright, S., 1946. Isolation by distance under diverse systems of mating. Genetics 31:39-59.

Wright, S., 1951. The genetical structure of populations. Ann. Eugen. 15:324-345.

Wright, S., 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 9:395-420.

CHAPTER 5. Laboratory studies on social structure and colony founding ability in two species of dolichoderine ants

INTRODUCTION

Most ant species have colonies founded by single queens (haplometrosis). However, in quite a few ant genera and species, colony founding by small groups of reproductive females (pleometrosis) is not uncommon. Laboratory studies on three species of ants (Waloff, 1957; Wilson, 1966; Hölldobler and Wilson, 1977) have demonstrated that group founding increases survival rate and decreases the time to maturity of the first brood.

In this study I have investigated the demography of laboratory colonies of two closely related species of dolichoderine ants, Conomyrma bicolor and C. insana. For C. bicolor, the effect of pleometrosis was examined. Although there is no published field evidence of multiple-queening in C. bicolor, partial excavations of C. bicolor nests have produced 3 to 7 queens; those of C. insana have not revealed more than a single dealate female (Roy R. Snelling, pers. comm.). In a study on the population genetics of three species of dolichoderine ants (Chapter 3), I have demonstrated that polygyny is the most probable state in C. bicolor field colonies, while monogyny is most likely in the C. insana colonies studied. This paper reports indirect evidence for naturally occurring polygyny in this species.

Colony rearing experiments were performed over a three year period. These experiments were designed to test the effect of queen number on colony founding ability. Six measures of colony founding ability were made under the various treatments: (1) the probability of egg laying, (2) the time to the laying of the first egg, (3) the maximum brood size,

(4) the time to maximum brood, (5) the maximum brood per female, and (6) the time to colony extinction (defined by the death of the last queen).

If colony success increases as a function of queen number, we can make predictions about the direction of change, as queen number increases, of the various indicators of success measured. The predictions tested in this paper are that, as queen number increases: (1) the likelihood of egg-laying increases, (2) the time to the laying of the first egg decreases, (3) maximum brood size increases, (4) the time to maximum brood size decreases, (5) the maximum brood per female increases, and (6) the time to colony extinction increases.

In this paper, I test these predictions in one polygynous species, Conomyrma bicolor, and compare the demography of laboratory colonies of bicolor with those of a monogynous congener, Conomyrma insana.

MATERIALS AND METHODS

Collections

In central Orange County, California, C. insana and C. bicolor alates swarm in the early spring. The days on which they swarm are characterized by being unusually calm and warm, following a period of rain. After mating, females drop out of the swarm to the ground, shed their wings and search for nest sites. While they were searching, these newly mated, wingless females were collected and brought into the laboratory to begin new colonies. Queens were collected in William R. Mason park (southeast corner of Culver Drive and University Drive) in Irvine, California in the follow quantities: 1975, 36 C. bicolor and 3 C. insana; 1976, 17 C. bicolor; and 1977, 114 C. bicolor. Females were placed individually in small vials. They were transported to the laboratory where they were set up in artificial nests within 24 hours.

Artificial Nests

Two styles of artificial nest were used. The simplest were comprised of 35 x 10 mm plastic Petri dishes (Falcon #1008) lined with filter paper. A small hole was made in the top of each nest into which a cotton wick for water was inserted. The bottom half of a second dish was inverted over the wick. This latter dish was lined with a damp sponge cloth circle which served to keep the wick moist for several days.

Larger nests were made from 30 ml plastic tissue culture flasks (Corning #25100, Falcon #3013). Each flask had a small plastic Petri

dish glued onto the top flat surface of the flask. The interior of the dish was connected to that of the flask by a hole about 7 mm in diameter. Moisture was provided via a cotton wick in the neck of the flask. Water could be added with a hypodermic needle via a small hole in the flask cap. Food, where provided, was placed in the Petri dish.

Nests were kept at room temperature, which was relatively constant, averaging about 20°C. Mite infestations, originally a problem, were controlled by paper towels which had been sprayed with benzyl benzoate, diluted 1:1 with 95% ethanol.

Experimental Design

In 1975, 15 small nests were set up. Animals were not provided with food, only with water. These conditions simulate those for founding females in the field. Except for a few primitive species, ant females are not known to forage during the early stages of nest founding. They feed their first brood on fat body and flight muscle reserves (Wilson, 1971).

Of the 15 nests, there were 4 with one C. bicolor each, 4 with 2 C. bicolor each, 4 with 4 C. bicolor each, 1 with 8 C. bicolor, 1 with 1 C. insana, and 1 with 2 C. insana. By the end of the third week, most of the C. bicolor queens had died. To test the hypothesis that their deaths were due to lack of sufficient food stores, 2 of the remaining 7 bicolor nests were provided with honey-water, while the other 5 were continued on water only. By the end of the fifth week, only the fed queens were alive. Although alive, these colonies had not grown during the two weeks they had been fed. Therefore, at this time,

Figure 1. Experimental design followed in manipulation of Conomyrma bicolor colonies in 1975.

DAY 1 - all colonies set up in small nests with water only	DAY 22 - those colonies followed by a * given honey-water; others only plain water	DAY 36 - remaining colonies moved to large nests, provided workers and food
1 queen.....died		
1 queen.....died		
1 queen.....died		
1 queen.....	1 queen, 0 eggs.....died	
2 queens.....died		
2 queens.....died		
2 queens.....	1 queen, 4 eggs*.....	1 queen, 3 eggs*
2 queens.....	1 queen, 4 eggs.....died	
4 queens.....died		
4 queens.....	2 queens, 4 eggs*.....	2 queens, 3 eggs*
4 queens.....	2 queens, 8 eggs.....died	
4 queens.....	2 queens, 9 eggs.....died	
8 queens.....	4 queens, 9 eggs.....died	

the two remaining bicolor colonies were moved into large nests where workers could be added to aid in feeding. At this time, one nest had one queen and 3 eggs, the other had 2 queens and 3 eggs. Workers were obtained from the same area as the queens and colonies were given one worker per queen. These nests were fed honey-water and dead adult Drosophila spp. The design of these experiments is diagrammed in Figure 1.

In 1976, 11 small nests were established: 4 had one reproductive each, 2 had two each, 1 had three, 2 nests had one queen and one worker each, and the last 2 had two queens and two workers each. None of the nests were fed. The nests containing workers as well as queens were intended to analyze the role of workers in initiating egg-laying and in rearing brood apart from the part they play as foragers for food.

Based on results from the 1975 and 1976 experiments, in 1977 a large scale experiment was designed to analyze the effects of number of queens, presence or absence of food, presence or absence of workers, and nest size on laboratory colony founding success. Thirty-eight small nests and 24 large nests were established. Of the small nests 14 had one reproductive each, 16 had two each, and 8 had four each. Of the large nests, 12 had one queen each and 12 had two each. Half of each category (for example, large nest, two queens) was provided with one worker per reproductive. Half of the large nests of each type were fed with honey-water and adult Drosophila spp. (See Tables 1 and 3 for a summary of the experimental design.)

Nests were checked twice a week. The numbers of eggs and larvae and their developmental stage were recorded. Nests were cleaned with a

small cotton swab when necessary, taking care not to disturb eggs and larvae. Fresh water and food (where appropriate) were provided at this time. From these data the six colony growth and success indicators listed in the Introduction were calculated for each colony.

RESULTS

Field Observations of Mating Flights

My field observations of Conomyrma bicolor at the time of swarming suggest that: (1) mating may be very localized and temporally patchy, (2) multiple-queening as a result of females being added to their natal or other existing colonies may not be uncommon, and (3) multiple insemination of females may take place.

C. bicolor, the principal subject of this study, was observed to swarm in the collecting area when air temperatures in the shade were between 21°C and 30°C. They avoid mid-day heat on exceptionally warm days. In the three years they were collected, mating swarms were observed as early as March 17 and as late as March 31. In any given year, the colonies at Mason Park swarmed over about a two week period. On any given day, alates from several colonies in one part of the park fly out and congregate around nearby trees or tall shrubs. There may be more than one local swarm on the same day within a few hundred feet of each other. On subsequent swarming days, alates from yet other colonies form the swarm(s).

In the course of collecting C. bicolor females, I observed 2 alate females emerge from a nest and then receive aid in shedding their wings from the workers of the nest. One of the females then re-entered the nest from which she emerged. Whether she was inseminated is not known. In Iridomyrmex humilis, winged females mate within the nest and then shed their wings (Markin, 1970). In Pseudomyrmex venefica (a polygynous, obligate acacia-ant) mating occurs on the branches of the

acacia occupied by the colony that produces the queens. Some of the newly mated females then re-enter thorns in their own colonies (Janzen, 1973).

I further observed, during one mating swarm, that many of the C. bicolor females landed near (within 3 to 6 meters) nests that were releasing alates that day. Some of the landing females were ushered into nests of bicolor by their workers; others were fought off by workers. I also observed attacks on dealate C. bicolor females by C. insana workers near the nest openings of the latter. One of these attacks was seen to be preceded by a bicolor female having entered an insana nest from which she was subsequently removed and dragged away by resident workers. Thus, it may be relatively common for mated females to attempt to enter existing nests. One way in which nest choice appears to be made is by the resident workers either accepting or rejecting the female. Since nest parasitism by bicolor on insana is not known to occur, it is assumed that these attempts were errors on the part of the reproductives.

On one occasion, swarming C. bicolor reproductives collected around my head and actually followed me while swarming. I had the unique opportunity of observing mating very closely. Many times after pairing with a male and falling to the ground with him, a female took flight and rejoined the swarm, suggesting the possibility of multiple insemination. An alternative explanation of this behavior would be a failure to complete copulation during many attempts.

Data from Artifical Nests

The pooled data from all three years indicate that single-queened, workerless C. bicolor colonies are much less likely to produce eggs than are all other colony types (one tailed p = .000019; Fisher exact probability; data pooled from 62 small nests). In 1975 and 1976, none of the single-queened, workerless colonies ever produced eggs. Conversely, of 16 colonies with more than one queen, or with one queen and one worker, all but two had eggs (one-tailed p = .00014, Fisher exact probability). However, in 1977, single-queened C. bicolor colonies did not differ significantly from all others (one-tailed p = .12, Fisher exact probability).

Although only two C. insana colonies were established, the data from these are very different from the C. bicolor data. Both colonies were effectively single-queened since the second female in the two-queen colony was dead by the second day of the experiment. The dead queen was dismembered and appeared to have been killed by its co-queen.

Values of the six indicators of colony growth and success in unfed, small nest colonies of C. bicolor and C. insana are summarized in Table 1. Both insana colonies produced large numbers of eggs, in marked contrast to single-queened C. bicolor colonies established simultaneously. Even compared with those bicolor colonies which laid eggs (1977), the rate of egg production in insana was greater (one-tailed $p < .01$, Mann-Whitney U).

If colony success increases as a function of queen number in C. bicolor, we might expect some of the growth and success measures to change accordingly. Table 2 summarizes the fit of the data to the

Table 1. Growth and survivorship data for newly founded laboratory colonies of Conomyrma bicolor and C. insana. The numbers of founding queens and workers are indicated and the values for six possible success indices are given. All colonies were reared without food.

Year	Colony time and type: Number of founding queens	Number of founding workers	Number of such colonies	Percent of colonies with eggs	Days* to first egg	Maximum brood size	Offspring* per queen at maximum	Days* to maximum brood	Days* to death of last queen
	*Conomyrma bicolor								
1975	1	0	4	0	-	0.0	0.0	-	23
	2	0	4	100	8	9.8	4.9	10	23
	4	0	4	100	8	14.0	3.5	11	25
	8	0	1	100	7	15.0	1.9	10	29
1976	1	0	4	0	-	0.0	0.0	-	12
	2	0	2	0	-	0.0	0.0	-	5
	3	0	1	100	5	9.0	3.0	10	24
1977	1	0	7	71	6	5.2	5.2	8	23
	2	0	8	100	6	15.1	7.6	8	29
	4	0	4	100	6	16.8	4.2	6	21
	1	1	7	71	6	4.8	4.8	7	23
	2	2	8	100	6	13.2	6.6	9	31
	4	4	4	100	6	34.5	8.6	7	31
	Conomyrma insana								
1975	1	0	1	100	11	25.0	25.0	48	147
	2	0	1	100	4	15.0	7.5	31	137

* Mean value in cases of more than one colony.

Table 2. Significance tests of the hypothesis (H_1) that colony and/or individual success increase in C. bicolor as the number of founding queens increases. For each index, colonies founded by 1, 2, and 4 queens are compared pairwise and the probability of no change with queen number (H_0) is given.

Predicted directional change in success index as numbers of founding females increases	Year	P values∞ for paired comparisons		
		1 vs. 2 females	1 vs. 4 females	2 vs. 4 females
Likelihood of egg-laying increases	1975-7	.01*	.01*	.39
	1975	.03*	.03*	-
	1977	>.05	>.05	-
Egg-laying begins earlier	1975	-	-	.56
	1977	.31	.37	.53
Maximum colony brood size increases	1975	.01*	.01*	.17
	1977	.01*	.01*	.14
Shorter time to maximum brood	1975	-	-	.40
	1977	.30	.21	.17
Brood per founding female increases	1975	.01*	.01*	.54
	1977	.14	.30	.04+
Last surviving queen lives longer	1975	.50	.40	.40
	1977	.10	.32	.03+

∞ P values for likelihood of egg-laying comparisons are Fisher exact probabilities; all other P values were obtained from U values in the Mann-Whitney test.
* One-tailed $P < .05$; change in predicted direction.
+ One-tailed $P < .05$; change in opposite direction to that predicted.

predicted changes. Taken as a whole, the data indicate that bicolor colonies with two to four queens are significantly more likely to have eggs than are single-queened colonies (one-tailed $p = .002$, Fisher exact probability). However, colonies with four queens are not significantly more likely to have eggs than are two-queened colonies. All data sets tested also showed that two- and four-queened colonies had significantly more eggs in total than did single-queened colonies with eggs (one-tailed $p < .01$, Mann-Whitney U). Again, there is no significant difference between two- and four-queened colonies. None of the other indicators (time to the laying of the first egg, time to maximum brood, maximum brood per female, or time to colony extinction) varied significantly with queen number.

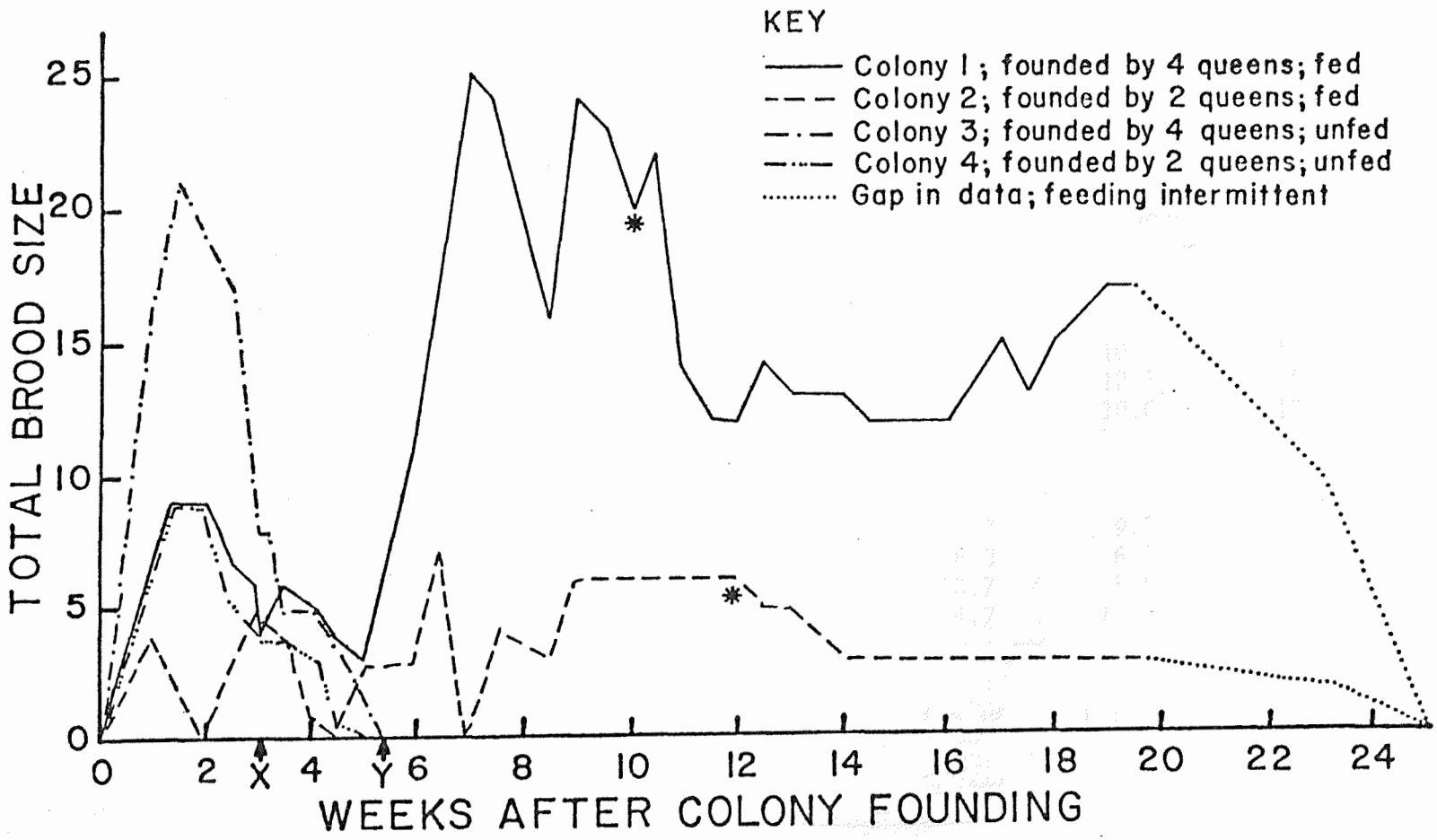
In 1975, the success of C. bicolor colonies sharply declined during the third week. This was in marked contrast to the two insana colonies which continued at a constant or increasing brood size until the ninth week when they were attacked by mites and fungus. It had already been observed that single queens of bicolor failed to lay eggs. This, combined with field observations of females being ushered into existing nests, suggested that C. bicolor females in Mason Park might begin their reproductive phase as members of established colonies. In such colonies, females could be fed by workers. Therefore, to test the hypothesis that C. bicolor reproduction required external food, I began to feed two of the remaining seven colonies. The two fed colonies survived and, ultimately, grew until the experiment was terminated at the beginning of the sixth month. In contrast, all unfed colonies were extinct by the end of the fifth week. Data from the

two fed colonies and two control, unfed colonies are illustrated in Figure 2. For the first two weeks following the onset of feeding, there was not much growth in the fed colonies. To examine the role which workers might play in mediating feeding, one worker per female was added to each nest while moving each colony into a larger nest with a separate feeding arena. Both colonies underwent growth spurts immediately following worker introduction.

I never succeeded in rearing workers from artificial nests. The nests all finally succumbed to mites or fungus. For each species examined, some colonies did produce brood which developed through several larval molts. Therefore, it is assumed that the laboratory environment established was minimally satisfactory to both species. During their early stages, the nests were relatively clean. Measures reflecting colony growth rate during this period are considered to be reliable. Also, since natural colonies do not occur under sterile conditions, the ability of a colony to persist in the presence of mites and fungus is considered as an indication of potential success.

In 1977, a study of the effect of feeding on colony growth and survival was undertaken. Colonies set up in large flask nests are easily fed. Half of each category of nests (for example, half of the single-queened workerless nests) was fed. The other half of each category nested under identical conditions, but without food. The same "success" indicators as were previously measured were quantified for these colonies. The null hypothesis that feeding had no effect on any of these measures of success was tested. These tests are summarized in Table 3. Four of six indicators were unaffected by

Figure 2. Sample colony growth data for two fed and two unfed *Conomyrma bicolor* colonies reared in the laboratory in 1975.



*= First larvae appear
X= Feeding initiated in Colonies 1 and 2
Y= One worker per queen added to Colonies 1 and 2

Table 3. Growth and survivorship of newly founded laboratory colonies of Conomyrma bicolor in the presence and absence of feeding. Mean values of three replicates of each colony type are given for each of six indicators. The predicted directional difference in indicators between fed and unfed colonies is indicated and the significance of the fit of the data to the prediction is given.

Colony type		Percent of		Maximum	Offspring*	Days* to	Days* to
Number of founding queens	Number of founding workers	colonies with eggs	Days* to first egg	brood size	per queen at maximum	maximum brood	death of last queen
Unfed colonies							
1	0	100	5	10.0	10.0	12.3	26
1	1	100	7	12.3	12.3	8.0	27
2	0	100	6	20.0	10.0	10.0	28
2	2	100	6	23.3	11.7	9.3	34
Fed colonies							
1	0	100	7	9.7	9.7	31.7	102
1	1	67	22	6.3	6.3	34.0	69
2	0	100	7	10.7	5.5	28.3	92
2	2	100	6	24.7	12.3	20.7	119
Predicted directional difference between fed (F) and unfed(UF), if feeding increases success		F > UF	F < UF	F > UF	F > UF	F < UF	F > UF
Fit of data to prediction‡		.50+	.06+	>.10+	>.10+	<.001+*	<.001*

‡ One-tailed probabilities; P values for the percent of colonies with eggs and days to first egg are Fisher exact probabilities; all other P values were obtained from U values in the Mann-Whitney test.
+ Difference not significant; direction opposite to that predicted.
+*Difference significant; direction opposite to that predicted.
* Difference significant in predicted direction.

feeding. One measure, length of time to the death of the last queen, was very different in the fed colonies as compared with unfed colonies. Fed colonies survived, on the average, longer than 95 days. Unfed colonies had an average survival time of 28.8 days. This difference is highly significant (one-tailed $p < .001$, Mann-Whitney U). A second indicator, time to maximum brood size, differed significantly between fed versus unfed colonies. In this case, the difference is in the opposite direction of that originally predicted and is highly significant (one-tailed $p < .001$, Mann-Whitney U). A closer inspection of the data reveals that most of the unfed colonies attained maximum brood sizes between days 8 and 10 and then rapidly declined. The fed colonies continued to grow for several more weeks. This difference then confirms the idea that food is necessary for persistence through the period of colony founding in C. bicolor. Among fed colonies, a comparison of workerless colonies with those having workers shows no systematic differences.

The data from artificial laboratory colonies corroborate with the observations made in the field during mating flights. In C. bicolor, single-queened colonies were less successful than were multi-queened colonies in two ways: (1) many fewer colonies produced eggs and (2) of those that did produce eggs, fewer eggs, in total, were laid in single-queened colonies than in multiple-queened colonies. There is a strong indication that the failure of single-queened colonies to lay eggs altogether is not absolute. It is likely that this tendency varies spatially and (or) temporally among colonies. If reproductive females of C. bicolor are fed during reproduction, as they probably

are if they join existing colonies, then the probability of surviving is greatly increased.

From the small sample size tested, C. insana females seem to be eminently capable of independent claustral colony founding. There is an indication that they may not tolerate multiple-queening, inasmuch as the only two-queened colony established was immediately reduced to a monogynous state.

DISCUSSION

The experiments reported in this paper were designed to test possible advantages of pleometrosis. In two of the three years studied, all monogynously founded Conomyrma bicolor laboratory colonies failed to produce eggs. In contrast, eighty-three percent of the multiple-queened laboratory colonies produced eggs. In a third year, the comparison was less extreme, with 71% of monogynous and 100% of polygynous colonies producing eggs. The second year, when no solitary females laid eggs, single queens accompanied by workers did lay eggs, suggesting that some interaction between the queen and other queen(s) and (or) worker(s) may be required before egg-laying is initiated by some females. Peacock (1950) noted a similar phenomenon with isolated queens of Monomorium pharaonis, a highly polygynous species.

The mating swarm from which females were collected the third year was much larger than those observed in either of the two preceding years. Perhaps the females collected during the first two years were spatially and (or) temporally peripheral to the main swarming activity. Alternatively, the number of colonies swarming during the third year may have increased due to population growth. Therefore, the colony founding behavior of newly mated females appears to be variable on a microgeographic scale.

In all years, colonies founded polygynously produced more eggs than did those founded by single queens. However, the number of eggs per female was not greater in polygynous colonies. Similar experiments on Lasius flavus, Camponotus vagus and Solenopsis invicta have shown

more rapid rates of brood development in polygynously founded colonies (Waloff, 1957; Stumper, 1962; Wilson, 1966).

Queen survivorship data strongly suggest that unfed bicolor females are too short-lived to found colonies successfully. Regardless of the number of queens per colony, no C. bicolor queen lived longer than 34 days without feeding. In contrast, C. insana queens lived up to 147 days without food. By the 34th day following egg-laying, even in the fastest growing colony, C. bicolor larvae had developed only to the second instar. (This colony was one which was fed and had one worker.) The most advanced developmental stage reached was the third larval instar, which required from 48 to 51 days from the day of egg-laying. In insana, where the time from egg-laying to the 3rd larval instar was 42-44 days, additional time to pupation was from 19 to 28 days. If development from the third larval instar to pupa in bicolor is similar to that in insana, a minimal estimate of the time from egg-laying to eclosion would be 67 days, almost twice the lifespan of the most long-lived unfed bicolor queens. The rearing conditions were probably not optimal. It is known that temperature can play a critical role in development time. Vanderplank (1960) found that in Oecophylla sp. (a weaver ant) the time taken from ovum to adult varied from 50 days at 24°C to 18 days at 30°C. At 16°C many eggs were laid, but none hatched.

It is possible that field reared colonies may experience warmer temperatures than those in the laboratory (18°-22°C). During the course of these experiments, the mean daily high temperature during the month of April was 17.9°C; average low was 10.7°C. In 1977,

the warmest year, the average high temperature was 20.6°C. Soil temperature near the surface may be up to 9°C higher than air temperatures at the hottest time of the day (Berkelhamer, unpubl. data). Therefore it is conceivable that in some years, bicolor females may be able to raise workers within the short time they are able to survive on their metabolic reserves alone. However, the lifespan of unfed bicolor females after mating is very short compared with that of females of species known to found colonies claustrally (Crawley and Donisthorpe, 1910; Vanderplank, 1960).

The failure of all unfed C. bicolor females to survive longer than 34 days is in marked contrast to the survival and renewed growth shown by fed colonies (Figure 2). That an upsurge in brood size depends not only on food but on the presence of workers is also evident from these data. Subsequent experiments designed to reveal the relative importance of food and workers (Table 3) revealed no significant difference between fed workerless colonies and those which were fed via workers, but the sample sizes were extremely small (3 replicates of each category). In addition, having been fed from the beginning, colony queens were relatively unstressed so differences may have been minimized. Under natural conditions foraging by queens is rare and doubtless involves considerable risk to the queens. Experiments which specifically tested the effect of feeding on colony growth and survival verified that queen survival was greatly increased by feeding. Survival was increased in all experimental colonies to a point where successful brood rearing was possible.

Therefore, it is strongly suggested that Conomyrma bicolor females from the Mason Park population studied have insufficient food reserves to found colonies claustrally. Waloff (1957) found high rates of queen mortality in Lasius flavus due to depletion of reserves in queens forced to found colonies independently. In Lasius flavus, colony founding is accomplished by groups of females, unaided by workers. This is probably not the situation in C. bicolor. Groups of unaided, unfed C. bicolor females have no better survivorship than do single females (Table 2). Either auxiliary queens must forage and feed each other, or colony founding must normally take place by queens accompanied by workers which feed the queens and their larvae. The failure of some fed, workerless colonies to grow (Figure 2) supports the latter hypothesis. Secondary pleometrosis is probably a frequent occurrence in this species, with new colonies formed by fission of older polygynous colonies. This method of colony reproduction has been described for other pleometrotic ants, for example, Monomorium pharaonis (Peacock et al., 1950), Iridomyrmex detectus (Duncan-Weatherly, 1953), Formica rufa (Elton, 1932), and F. polyctena (Gösswald, 1951). In I. detectus, another dolichoderine species, new colonies begin as offshoots from the parent colony and subsequently become independent.

The field observations reported in this chapter corroborate both the laboratory experiments and the genetic evidence (Chapter 3) concerning the species studied. The genetic analysis indicates that, in the populations studied, polygyny is common for C. bicolor while monogyny is typical of C. insana. By contrast, Nickerson et al. (1975)

have convincingly demonstrated that colonies of a Florida population of C. insana are polygynous. In Florida, C. insana is rare and is replaced by its congener C. flavopectus (Creighton, 1950). It is possible that the Florida and California populations represent different subspecies or even two different species that have not been distinguished. The taxonomic status of C. insana in the western United States is currently in flux. Alternatively, polygyny may be a locally advantageous system in some populations of C. insana and not in others. Thus, the species may show behavioral variability over a broad geographic range.

Conomyrma bicolor has a much narrower geographic distribution than C. insana. It is restricted to arid regions of the southwestern United States and northwestern Mexico. In these areas it often occurs microsympatrically with C. insana, which has a broader distribution occurring all along the west coast of the United States. Of nine populations of bicolor examined, ranging from coastal southern California (Irvine) to eighty kilometers inland (Warner Springs, Ca.), electrophoretic evidence indicates that all but one of the populations included some colonies that could not have been produced by only one, singly-inseminated female. There is a weak relationship between the frequency of polygynous colonies and the proximity of human dwellings; polygyny is commoner within 1 km of habitation ($p = .09$, Mann-Whitney U; Table 4.).

According to Mallis (1941), Conomyrma bicolor was an annoying house ant in Southern California prior to the introduction and subsequent spread of the Argentine Ant, Iridomyrmex humilis. The close

Table 4. Probable occurrence of polygyny and distance from human habitation at 9 localities. Distance from habitation is given as greater or less than 1 km to indicate very close proximity versus some distance. A minimum estimate of the occurrence of polygyny (and/or multiple insemination without sperm precedence) is given by the percent of sampled colonies which could not have been produced by single queens inseminated once. Data are based on genotype frequencies at the GOT-2 locus.

Site	Distance to human habitations	Percent of colonies which cannot be from single females inseminated once-GOT-2 data
A	>1 km	75
B	>1 km	27
D	>1 km	25
F	<1 km	100
Palomar (P)	>1 km	0
Irvine (I)	<1 km	58
Warner (W)	<1 km	100
Ortega (O)	>1 km	100
El Toro (ET)	<1 km	100

H_0: There is no difference in the 'percent' between colonies less than 1 km and more than 1 km from human habitation.

H_A: There is a higher 'percent' among colonies less than 1 km from habitation.

One-tailed $p < .09$, Mann-Whitney U test.

A, B, D, and F are localities in the valley to the east of Palomar Mountain, San Diego County, California.

See Chapter 2 for exact locality data.

association of humans and Argentine Ants in southern California was noted over forty years ago by Eckert and Mallis (1937). Although once very common near human settlement, C. bicolor may now be restricted in these areas to small habitat patches which are still less suitable for Iridomyrmex humilis than for C. bicolor.

One possible explanation for the observed geographic variation in reproductive habits of C. bicolor and C. insana is that polygyny may be favored in marginal or restricted habitats (such as the edge of the geographical range of C. insana, or, in the cases of bicolor, in habitats that have undergone severe human alteration). Some ant species that are regularly highly polygynous also occur in very restricted habitats. Janzen (1973) noted that polygynous species of obligate acacia-ants (Pseudomyrmex spp.) are found in only two parts of the range of swollen thorn acacias. Although ecologically very different, these two parts of the range are both very atypical with respect to most swollen-thorn acacias. Over most of their range, swollen-thorn acacias are occupied by monogynous species of acacia-ants. Similarly, polygynous mound-building ant species, Formica ulkei, has a widely scattered distribution in the Chicago, Illinois area. Within this region it appears to be restricted to forest margin habitats (Dreyer and Park, 1932; Scherba, 1958).

There are many ways in which polygyny may be adapative to species living in restricted or marginal habitats. The advantages fall into two main categories: those affecting colony founding and those affecting the growth and ultimately the production of reproductives in established colonies. It has been the object of this paper to

deal with the former. The experiments reported here have strongly supported the idea that polygyny, especially when coupled with the presence of workers, may decrease the likelihood of extinction of founding colonies, a threat that may pose especially severe limitations in restricted or marginal habitats.

Due to their limited nature, such habitats may support relatively small populations and polygyny may be one way of maintaining higher effective population sizes than these habitats would support on a one queen per colony basis. Wilson (1963) proposed a similar hypothesis to explain the relatively greater frequency of polygyny among rare ant species than among closely related common species. He suggested that due to their rareness, these species may have evolved polygyny as a means of increasing effective population size and, thus, reducing the danger of extinction. Later, Wilson (1966) proposed an alternative explanation in terms of the kin selection hypothesis for the evolution of sterile worker castes in the eusocial Hymenoptera (Hamilton, 1964). According to this second hypothesis, the small number of individuals in these populations of rare ants results in a relatively high average genetic relatedness amongst associated queens which predisposes queens towards altruistic cooperation. Hamilton (1972) offers a similar explanation for polygyny on a much broader scale. The application of the genetic hypothesis to Conomyrma insana, C. bicolor and a third species, Iridomyrmex pruinosum, is examined in Chapter 4.

CONCLUSIONS

This study reports the following:

1. Field observations indicate that Conomyrma bicolor is polygynous while C. insana is monogynous. The field behavior of newly mated bicolor females suggests that polygyny may be achieved by incorporating new queens into existing colonies (secondary pleometrosis).
2. Laboratory colonies of Conomyrma insana develop as one would expect for a monogynous species. Solitary females are capable of producing and rearing brood solely on stored food reserves. Supernumary colony queens may be killed.
3. Conomyrma bicolor laboratory colonies exhibit very different developmental patterns from those of C. insana. Solitary females often do not lay eggs, while grouped females rarely fail. Polygynously founded colonies have significantly more eggs than do those founded monogynously. Unfed queens are very short-lived and appear unable to feed and rear brood by relying only on reserves. Feeding of queens during colony founding appears to be necessary in bicolor.
4. The frequency of polygyny in C. bicolor may vary temporally and (or) spatially on a microgeographic scale. In C. insana the same kind of variation may occur macrogeographically. Comparisons of patterns of variation in Conomyrma with the occurrence of polygyny in distantly related ant genera leads to the hypothesis that polygyny is associated with restricted or marginal habitats.

LITERATURE CITED

Crawley, W. C. and H. Donisthorpe, 1910. The founding of colonies by queen ants. Trans. Second Intern. Congr. Entomol., Oxford, pp. 11-77.

Creighton, W. S., 1950. Ants of North America. Bull. Mus. Comp. Zool. Harvard 104:585 pp.

Dreyer, W. A. and T. Park, 1932. Local distribution of Formica ulkei mound nests with reference to certain ecological factors. Psyche 39:127-133.

Duncan-Weatherly, A. H., 1953. Some aspects of the biology of the mound ant Iridomyrmex detectus (Smith). Australian J. Zool. 1:178-192.

Eckert, J. E. and A. Mallis, 1937. Ants and their control in California. Cal. Agric. Exp. Sta. Circ. 342:39 pp.

Elton, C. S., 1932. Territory among wood ants (Formica rufa L.) at Picket Hill. J. Anim. Ecol. 1:69-76.

Gösswald, K., 1951. Über den Lebensablauf von Kolonien der Roten Waldameise. Zool. Jb. 80:27-63.

Hamilton, W. D., 1964. The genetical evolution of social behavior. I and II. J. Theor. Biol. 7:1-52.

Hamilton, W. D., 1972. Altruism and related phenomena, mainly in social insects. Ann. Rev. Ecol. Syst. 3:193-232.

Hölldobler, B. and E. O. Wilson, 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8-15.

Janzen, D. H., 1973. Evolution of polygynous obligate acacia-ants in Western Mexico. J. Anim. Ecol. 42:727-750.

Mallis, A., 1941. A list of the ants of California with notes on their habits and distribution. Bull. So. Calif. Acad. Sci. 40:61-100.

Markin, G. P., 1970. The seasonal life cycle of the Argentine ant, Iridomyrmex humilis (Hymenoptera: Formicidae), in Southern California. Ann. Entomol. Soc. Am. 63:1238-1242.

Nickerson, J. C., H. L. Cromroy, W. H. Whitcomb, and J. A. Cornell, 1975. Colony organization and queen numbers in two species of Conomyrma. Ann. Entomol. Soc. Am. 68:1083-1085.

Peacock, A. D., 1950. Studies in Pharoah's ant, Monomorium pharaonis (L.) (4) Egg-production. Entomol. Mon. Mg. 86:294-298.

Peacock, A. D., D. W. Hall, I. C. Smith, and A. Goodfellow, 1950. The biology and control of the ant pest Monomorium pharaonis (L.). Dept. Agric. Scotland, Misc. Publ. 17:51 pp.

Scherba, G., 1958. Reproduction, nest orientation and population structure of an aggregation of mound nests of Formica ulkei Emery (Formicidae). Ins. Soc. 5:201-213.

Stumper, R., 1962. Sur un effet de groupe chez les femelles de Camponotus vagus (Scopoli). Ins. Soc. 9:329-333.

Vanderplank, F. L., 1960. The bionomics and ecology of the red tree ant, Oecophylla sp., and its relationship to the coconut bug Pseudotheraptus wayi Brown (Coreidae). J. Anim. Ecol. 29:15-33.

Waloff, N., 1957. The effect of the number of queens of the ant Lasius flavus (Fab.) (Hym., Formicidae) on their survival and on the rate of development of the first brood. Ins. Soc. 4: 391-408.

Wilson, E. O., 1963. Social modifications related to rareness in ant species. Evolution 17:249-253.

Wilson, E. O., 1966. Behaviour of social insects. Symposium Roy. Entomol. Soc. London 3:81-96.

Wilson, E. O., 1971. The Insect Societies. Belknap Press, Harvard Univ. Press, Cambridge, 548 pp.

CHAPTER 6. Ant activity following chaparral fire

INTRODUCTION

Fire is an important factor affecting chaparral communitites in California and is recognized to be the primary cause of secondary plant succession in chaparral habitats (Hanes, 1971). Although few mobile or burrowing animals are directly killed by fire (Lawrence, 1966; Gillon, 1971a, 1971b; Main, 1980), numbers are often indirectly altered by fire as a consequence of changes in predation levels, exposure to environmental extremes, and availability of food. Some species respond favorably to these changes, while others do not. For example, Lawrence (1966) found that numbers of predatory birds and mammals increased, as did those of some seed-eating birds, while populations of most small mammals and some brush-dwelling birds decreased. He attributed the observed density decreases to predation and the increases to increased food availability. Thus, the effect of fire on a species will be determined by a variety of factors, including availability of refugia during fire, tolerance for environmental extremes which may be experienced post-fire, ability to withstand increased vulnerability to predators after fire, and availability of suitable food post-fire.

Ants are particularly interesting animals in which to assess the effects of chaparral fire. They are a major component of the invertebrate fauna of chaparral communities. Hunt (1973) found 45 species of ants within an area of chaparral approximately .5 km^2. In addition to their diversity and abundance, ants are interesting because they vary with respect to social structure in ways that might be expected to have profound consequences for colonizing ability, an important factor in frequently disturbed habitats. Some ant species

are monogynous (have only one queen per colony) and begin colonies once a year following mating flights. Other, polygynous, species have many queens per colony and are able to form new colonies by "budding" from large, older colonies. This study documents responses by ants, particularly of the subfamily Dolichoderinae, to chaparral fire. It investigates the relationship between social structure and recovery after fire.

At Hunt's (1973) chaparral study site, two of the three most active species at baits were members of the subfamily Dolichoderinae. Taken together, five species of dolichoderine ants accounted for 47% of the activity at all baits and 53% at honey baits. Among the five common dolichoderine species, there is considerable variation in social structure. Iridomyrmex pruinosum, Conomyrma bicolor, and Tapinoma sessile are polygynous and probably able to reproduce by budding (Chapters 3 and 5; also Wheeler and Wheeler, 1973). Conomyrma insana is monogynous (Chapter 3); and Liometopum occidentale is probably monogynous (Wheeler, 1905). Even if L. occidentale is found to be occasionally multiple-queened, reproduction is not typically by budding. A sixth dolichoderine, Forelius foetida, is rare locally.

Polygynous ant species that are able to reproduce by colony fission should be at an advantage in habitats which are frequently disturbed by fire to the extent that new habitat openings may be colonized immediately. In contrast, monogynous species are not able to colonize until shortly after mating, a seasonal event. Most monogynous species found colonies claustrally (queens are unaccompanied by workers). Founding females do not forage and, instead, feed their

first brood solely on their own reserves (Wilson, 1971). It would take a minimum of several months for such a colony to have a worker force the size of that with which a budding species begins colonization. The lags inherent in claustral colony founding are a major factor leading to the prediction that claustral species will be poorer early colonizers following chaparral fire than polygynous, budding species.

In addition, claustrally founded colonies must be extremely vulnerable during the early stages, before the first brood are able to forage and help care for subsequent broods. Therefore, species which found claustrally might be expected to have a greater incidence of colonizing failures per colonizing attempt as compared with species which reproduce by fission. Ultimately, this must be balanced by the differential cost of the two modes of colonization. Although risky, claustral founding is relatively inexpensive (energetically) to the parent colony; whereas reproduction by budding is less risky but requires more energy per colonizing propagule. Claustral species would, thus, be expected to send out more colonizing propagules and to disperse greater distances. In the end, claustral species would be expected, stochastically, to find and colonize new habitat openings. However, they are expected to lag behind polygynous, budding species. The exact pattern would depend on discontinuities of suitable new habitat.

Therefore, it is predicted that the proportion of polygynous, budding species will increase shortly after fire, while that of monogynous species will decrease due to immediate colonization by polygynous species. This prediction assumes that there is no relationship between fire survival and the number of queens per colony.

After a period of time has passed, monogynous species are expected to become more numerous. As monogynous species colonize, species diversity is expected to increase over immediate post-fire levels. Timing of any decline in dominance by polygynous species would be dependent on the demography and life history of the species involved.

The contrast between claustral colony reproduction and reproduction by fission is somewhat analogous to that between chaparral plants which reproduce primarily via seeds and those which reproduce vegetatively from root crown sprouts. Hard chaparral, historically exposed to frequent burning, contains a large proportion of crown-sprouting species (Hanes, 1971). We might, similarly, expect a predominance of fission-reproducing ant species. The analogy is not perfect, however. Although the ant and plant strategies show the same contrast between colonists with rapid growth and those with delayed growth, the root-crown sprouting plant has an advantage in locating the fire area (it is already there) which the fission-reproducing ant may lack.

Shortly after fire, total ant activity might be expected to decrease due to lowered availability of food. Although dolichoderines are scavengers, they depend on other insects for most of their food; they eat dead insects and gather honeydew from homopterans. Immediately after fire, habitats and food for such insects are unavailable. However, in situations where root-crown sprouting is common, fresh sprouts occur very soon after fire. In this study, sprouts up to six inches tall grew in the first month following fire. Compared with old chaparral stands, recently burned areas have much higher growth rates of both shrubs and herbs. A recent study (Rundel and Parsons, 1980) has shown that this

new growth may be of higher nutritional value for herbivores. Such new growth, thus, should provide a relatively rich food source for homopterous insects and a host of herbivorous insects which, in turn, would provide food for ants.

Therefore, it is predicted that after an immediate post-fire dip in ant activity, there should be an increase in total ant activity due to increased food availabilty. This prediction, along with predictions of species composition and diversity based on social structure were tested in the five common dolichoderine species, two common formicines, and one myrmecine following a chaparral fire near Hot Springs Mountain in inland San Diego County, California.

MATERIALS AND METHODS

Ant activity was censused periodically following a small chaparral fire near Hot Springs Mountain, San Diego County, California. The exact location of the fire was 6.9 km N and .5 km W of Warner Springs, along Lost Valley Road, California 9SO2 (see Chapter 2, Figure 2, site B). The fire occurred in early May, 1974, and burned a total of 11 hectares on two adjacent hillsides. Censusing was principally on the more gradual slope which faced ENE and extended from 1280 to 1340 M (4200 to 4400 ft).

Ant nest occurrence and foraging activity were first censused 10 days after the fire. Activity was then censused periodically over the next 53 months. Nests were located by extensive surveying and intensive examination within meter-square quadrats. This procedure was important for baseline data on species present immediately after fire when foraging was extremely infrequent. However, nest locating was found to be destructive to the colonies uncovered. Therefore, later censusing was limited to measures of ant activity at honey-water baits which attracted all the locally apparent dolichoderine ants. Meat and peanut butter baits were also used initially, but these did not attract any ants which were not already attracted to the honey-water. Baits were set at 4 meter intervals along two transects across a relatively flat hilltop. One of the transects was in the burned and one in adjacent unburned chaparral. Baits were censused periodically throughout the day and renewed as needed. Baiting was discontinued when activity levels decreased in the evening and midday (summertime). All of the species studied are diurnal foragers. In many instance colony openings were

located by following foragers.

The activity level of a species was quantified as its percent occurrence at baits. That is, a species present at 6 of 30 baits would have an activity level of 20%. The total activity at baits was then found by summing the activity levels of all species. Therefore, total activity could exceed 100% when some baits attracted more than one ant species.

Air and soil temperatures were recorded throughout the sampling period. Air temperature was measured 5 cm above the ground in an unshaded area. During the measurement, the thermometer bulb was hand shaded to prevent heating of the bulb by direct solar radiation. Soil surface temperatures were measured in both sun and shade; again the bulb was shielded from the sun.

Critical thermal maximum (CTM) temperatures were measured in the laboratory for Conomyrma bicolor and Iridomyrmex pruinosum. For each trial, eight individuals of each species were tested; four at 100% humidity, four at 0% humidity. Temperature was regulated using a water bath in which air and water-tight Vacutainer test tubes were submersed. Each ant was in a separate test tube and temperature was recorded with a thermister probe inserted through the rubber stopper into a test tube which was exposed to the same treatment as the ants. One hundred percent relative humidity was attained with a moist cotton wick, zero percent with a small packet of Drierite. The wick and the Drierite were placed in the bottom of the test tubes.

Temperature was gradually increased until the CTM was approached. The rate of increase was about 1° to 2° C every 10 minutes. When the

CTM was approximated, new animals were then run at a series of temperatures (46° C, 47° C, 48° C, and 49° C) around the observed CTM. At each temperature, animal were observed until 50% of the animals exhibited a loss of the righting response, or until one hour had passed, whichever was shorter. Control animals in similar containers at room temperature exhibited no loss of righting response in an hour. In this way, estimates of CTM were refined and the effects of long trials and prior experimenting were eliminated. For each species, final CTM determinations are based on data from four animals for each humidity regime. For this study the CTM reprted is that temperature at which 50% of the animals showed a loss of righting response within 15 minutes of exposure.

RESULTS

The most conspicuous species in the burned area throughout the study was Iridomyrmex pruinosum, although it was not observed there in May, 1974, immediately following the fire. Dolichoderines as a group, were the most abundant ants at baits, accounting for an average of 60% of the ant activity in both the burned and the control areas. Patterns of total activity and species specific activity were analyzed separately.

Total Activity

Two hypotheses about total ant activity were tested. These were:

1. Ant activity, as measured by presence at baits, is greater following chaparral fire than before.
2. Species diversity of ants decreases immediately following chaparral fire, and then increases again.

Both of these hypotheses are weakly supported by the data.

The hypothesis that total ant activity increases following chaparral fire was tested using activity data from burned and control areas (Table 1). There is a nearly significant difference in the predicted direction (one-tailed $p = .0547$, Wilcoxon matched-pairs signed-ranks test). Within the burned area, there is a significant positive correlation between total activity at baits and time since fire (Kendall's $\tau = .500$, $p < .05$). To lessen the effect of seasonality patterns within these data, maximum air temperature was partialled out. The resulting partial correlation coefficient between total activity and

Table 1. Total ant activity, species diversity, species richness, species evenness, and Iridomyrmex pruinosum (I. p.) activity in burned (b) versus control (c) areas.

Date	Months Since Fire	Total Ant Activity*		Species Diversity**		Species Richness***		Species Evenness****		I. p. Activity*	
		b	c	b	c	b	c	b	c	b	c
10-74	5	131	44	1.60	1.04	8	3	.77	.95	59	0
7-75	14	127	150	1.20	1.82	9	7	.55	.93	69	30
7-76	26	85	90	.80	1.36	4	5	.58	.84	90	5
10-77	41	210	115	1.79	1.69	8	7	.86	.87	65	30
5-78	48	180	135	2.01	1.60	9	6	.91	.89	30	25
7-78	50	147	128	1.73	1.40	8	5	.83	.87	57	56
10-78	53	160	132	1.56	1.37	7	5	.80	.85	67	42
p(b=c)+		p = .0547		p = .40		p = .0546		p = .0468		p = .0078	

* Activity is expressed as percent occupation of baits. Activity levels greater than 100 indicate an average of more than one species per bait.

** Species diversity was calculated as the Shannon-Wiener information measure, $H = - \sum_i p_i \ln p_i$.

*** Species Richness was s, the number of species.

**** Species Evenness was calculated as $E = H/H_{max}$, where $H_{max} = -\ln s$.

\+ Probabilities were based on the likelihood of obtaining values as extreme as those found using the Wilcoxon matched-pairs signed-ranks test. For Species Evenness, there was no directional prediction, so the two-tailed probability is given. In all other cases, the prediction was b > c, and probabilities are one-tailed.

time since fire is nearly as great as the total correlation coefficient (Kendall's partial τ = .44, no probabilities available).

When samples from both the burn and the control are pooled, there is a significant correlation between total activity and Iridomyrmex pruinosum activity (Kendall's τ = .306, $p < .05$). However, there is not a similar trend within either the burn or the non-burn samples. This suggests that the activity difference observed between areas may be a reflection of differences in Iridomyrmex activity.

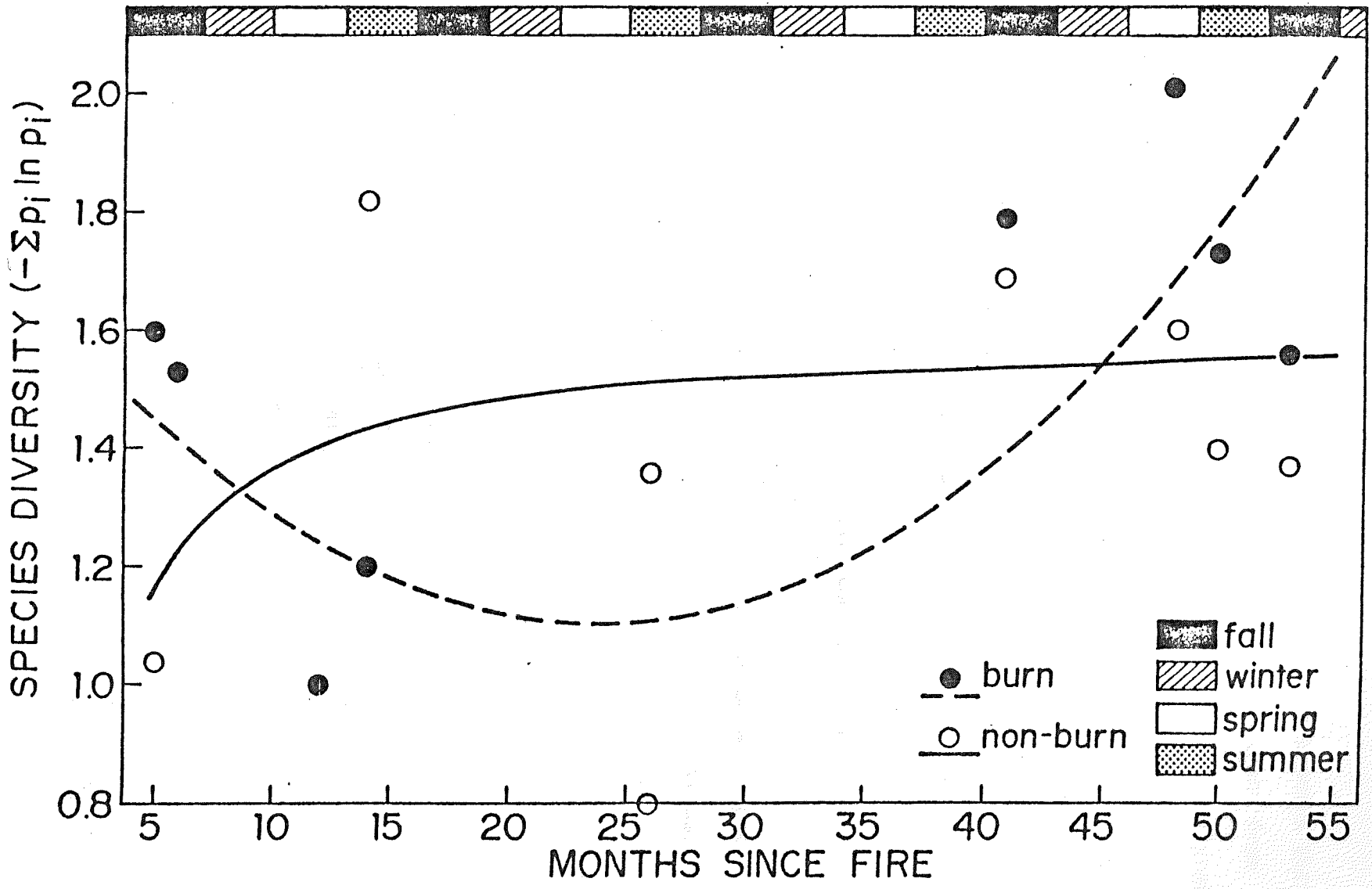
The trend in total ant activity is not mirrored by a similar pattern in species diversity (one-tailed $p >> .05$, Wilcoxon matched-pairs signed-ranks test, Table 1). Instead, as predicted, the pattern within the burn is for diversity to first drop and then climb. Before examining the exact nature of this and other species diversity patterns it is necessary to test for the possibility that the correlations and trends observed are not a spurious consequence of variations in sample size among samples. Therefore, the relationship between number of baits and species diversity was analyzed. Species-area studies (for example Wilson, 1961; Preston, 1962) suggest that we might expect a positive correlation between species diversity and sample size (number of baits). In fact, the relationship is negative and non-significant (Kendall's τ = -.122, $p > .05$, Table 2). Therefore, it may be assumed that sample size did not determine the observed species diversity relationships (Table 2 and Figures 1-3).

When species diversity is analyzed as a function of time (Table 2 and Figure 1), there are no overall trends within either the burn or control areas (burn: Kendall's τ = .250, $p > .05$; control: Kendall's

Table 2. Correlates of species diversity. In all cases species diversity is the Shannon-Weiner information measure, $H = -\sum_i p_i \ln p_i$.

Correlate	Correlated?	Kendall's τ	p
Number of baits used to sample diversity	NO	-.122	>.05
Total ant activity at baits (burn and control data pooled)	YES	.555	<.005
Iridomyrmex pruinosum activity at baits			
Burn and control pooled	NO	-.050	>.05
Burn alone	NO	-.333	>.05
Control alone	NO	.390	>.05
Time since fire - whole period			
Burn	NO	.250	>.05
Control	NO	-.048	>.05
Time since fire - two phases			
Burn			
First 26 months	YES	-.800	<.05
Next 27 months	NO	.000	>.05
First 26 months with *Iridomyrmex pruinosum* activity partialled out	YES	-.764	
Control			
First 26 months	NO	.333	>.05
Next 27 months	NO	-.200	>.05

Figure 1. Species diversity through time. The Shannon-Weiner information measure of diversity ($H = -\sum_i p_i \ln p_i$) is plotted as a function of time (in months) since the fire. Data from the burned area (•) shows a different pattern from that from the control, unburned area (o). In the burned area, species diversity shows two phases. During the first 26 months, species diversity declines. By 41 months after the fire, species diversity has climbed above early post-fire levels and appears to have plateaued. Curvilinear regressions which best explain the variance in species diversity within sites have been plotted to emphasize the two-phased nature of the pattern within the burn as compared with the more nearly linear pattern in the control.


SPECIES DIVERSITY ($-\Sigma p_i \ln p_i$)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
5
10
15
20
25
30
35
40
45
50
55
MONTHS SINCE FIRE
burn
non-burn
fall
winter
spring
summer

τ = -.048, p >> .05). Upon closer examination, however, it appears that the first two years after the fire may be qualitatively different from the next two years. When the burn data through July, 1976, are analyzed alone, there is a significant negative relationship between species diversity and time since the fire (Kendall's τ = -.800, p = .042). This relationship appears to be relatively independent of the negative correlation between Iridomyrmex activity and species diversity (Kendall's partial τ = -.764). Since July, 1976, species diversity appears to have plateaued and shows no correlation with time since the fire (Kendall's τ = .000, p >> .05). Species diversity within the control area does not show a similar two-phase pattern.

The curves which have been fit to the relationship between species diversity and time since fire (Figure 1) serve to graphically illustrate the difference between the burned and unburned areas. The fit of a variety of curvilinear models to the data was tested. For the data from the burned area, an upward opening parabola explained more of the variance in data (r^2 = .51, p = .12) than any other tested, including a linear model (r^2 = .25, p = .17). For the unburned chaparral, a hyperbolic curve provided the best fit (r^2 = .34, p = .17). Although these curves are not intended to imply a long term parabolic or hyperbolic relationship between species diversity and time, they do help us envision short term responses to perturbation in the two areas. In the burn, we see a fall and subsequent rise, as predicted. In the non-burn, an immediate rise is followed by a steady state. The short term rise may be due to a secondary low level invasion of the unburned chaparral by Iridomyrmex pruinosum, a previously uncommon species,

following its colonization of the nearby burned area. Iridomyrmex nesting near the burn edge were frequently attracted to non-burn baits near the edge. In general, then, species diversity patterns following fire are as predicted.

In addition to examining patterns in species diversity (H), it is instructive to look at the behavior of the two components of diversity, species richness (s = number of species) and species evenness ($E = H/H_{max}$, where $H_{max} = -\ln s$). Values for both of these are included in Table 1. When species evenness is plotted as a function of time since fire (Figure 2), there is a dramatic difference between the burn and non-burn sites. During the first phase, when diversity is falling, evenness is quite low in the burn as compared with the non-burn. The one exception to this is for the twelfth month burn sample which had an unusually low number of species active and, hence, a relatively high evenness value. In contrast, during the second half of the study (months 41 to 53), the species evenness in burn and non-burn areas are indistinguishable.

An examination of species richness through time (Figure 3) does not reveal the same two-phase pattern as do both species diversity and species evenness. Overall, however, species richness is greater in the burn than in the non-burn (two-tailed $p = .0468$, Wilcoxon matched-pairs signed-ranks test). The observed fit to the predicted species diversity pattern appear, therefore, to be predominantly due to low species evenness shortly after fire, as a consequence of the dominance of a few of the species present.

Figure 2. Species evenness through time. Species evenness, $E = H / H_{max}$, where $H_{max} = - \ln s$ (s = the number of species), is plotted against time, in months, since fire for both burned and unburned, control sites. As in the case of species diversity, species evenness shows a two-phase pattern within the burn, particularly when compared with the control. During the first 26 months, species evenness is much lower in the burn than in the control. From month 41 through 53, however, the evenness in the burn is indistinguishable from that in the control. The linear regression for control data, $y = .94 - .0016 x$, has been plotted to aid in visualization of the two phases in the burned area. This regression line provides quite a good fit to the control data ($r^2 = .5238$, $p = .066$).

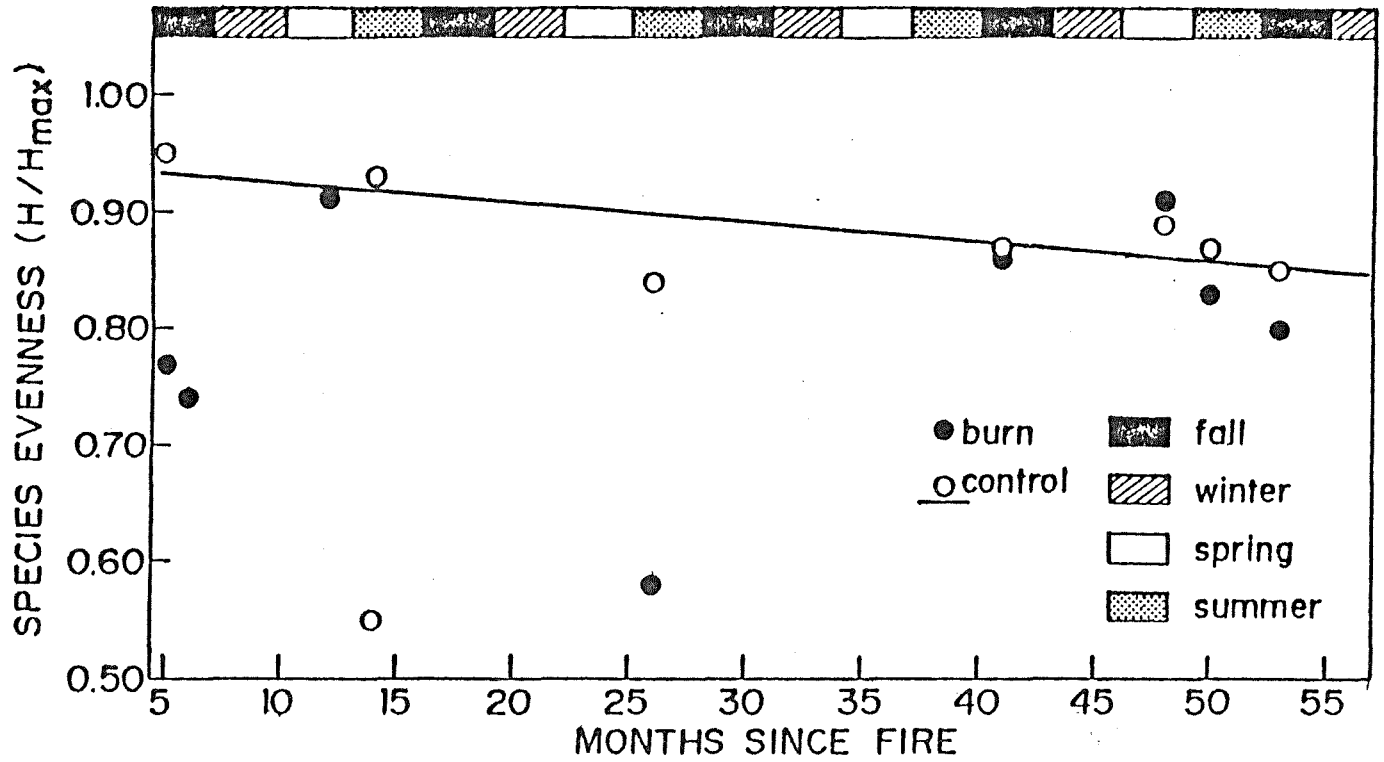
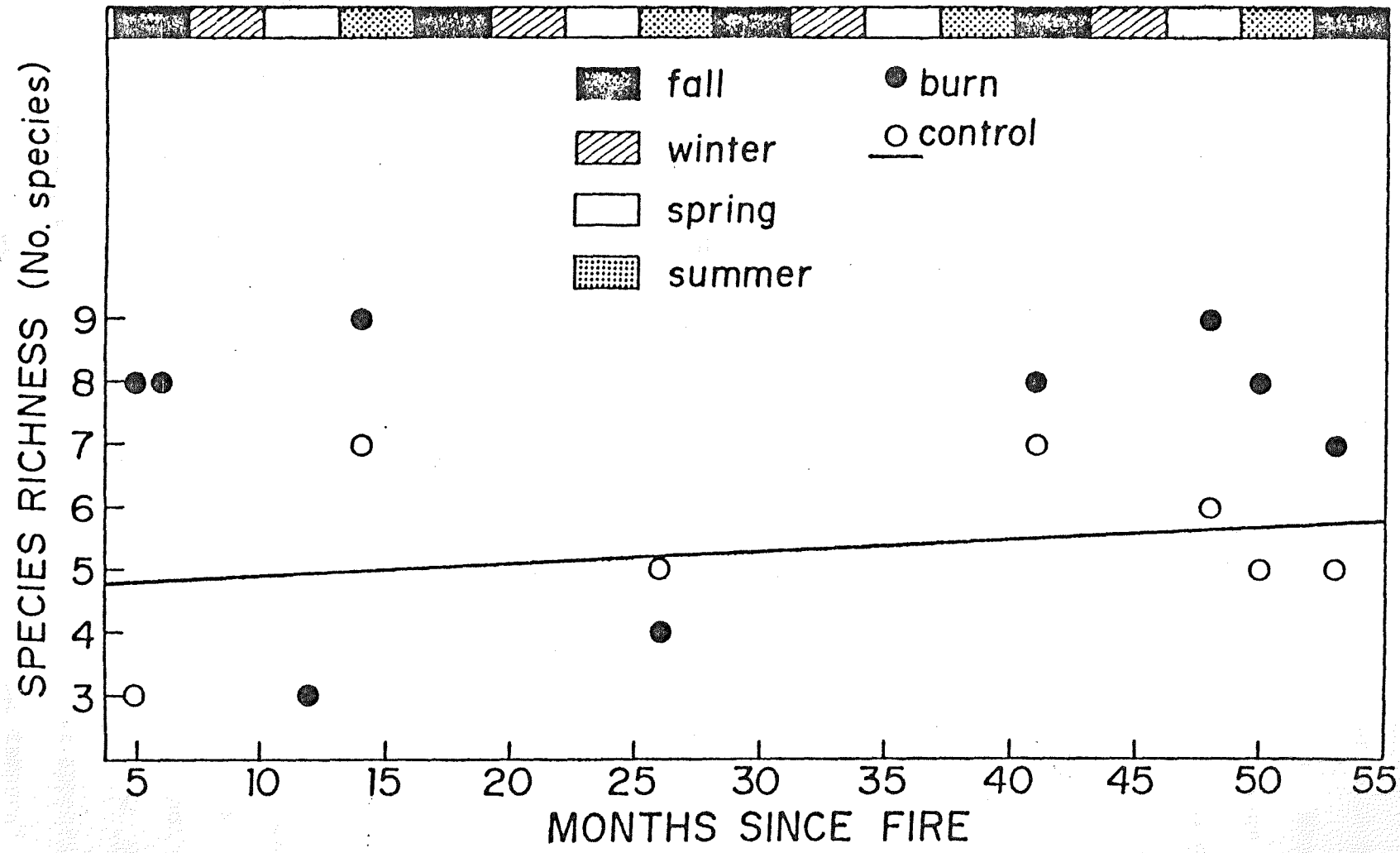

SPECIES EVENNESS (H/Hmax)
1.00
0.90
0.80
0.70
0.60
0.50
5
10
15
20
25
30
35
40
45
50
55
MONTHS SINCE FIRE
burn
control
fall
winter
spring
summer

Figure 3. Species richness through time. Species richness, s = the number of species, is plotted against time. There is no evidence of a two-phase pattern within the burn. The regression line, $y = 4.70 + .02\ x$, is drawn to help visualize the relationship between the control and burn data. This regression is based on the seven control samples and explains relatively little of the variance among samples ($r^2 = .08$, $p = .52$). Species richness is generally higher in the burned area than in the unburned area.


fall
winter
spring
summer
burn
control
SPECIES RICHNESS (No. species)
3
4
5
6
7
8
9
5
10
15
20
25
30
35
40
45
50
55
MONTHS SINCE FIRE

Not surprisingly, species diversity is highly correlated with total ant activity (Kendall's $\tau = .55$, one-tailed $p < .005$, Table 2). When all samples are analyzed together, there is no relationship between diversity and activity of the most active species, Iridomyrmex pruinosum (Kendall's $\tau = -.050$, $p >> .05$, Table 2). However, when burn and control samples are analyzed separately, the trends are strikingly different, although neither is significant. Within the control, species diversity is positively correlated with Iridomyrmex activity (Kendall's $\tau = .390$, $p > .05$). By contrast, in the burned area, species diversity is negatively correlated with Iridomyrmex activity (Kendall's $\tau = -.333$, $p > .05$).

Iridomyrmex pruinosum Activity

Based on a knowledge of its polygynous social structure, I predicted that Iridomyrmex activity would increase following fire and subsequently decrease to levels seen in unburned chaparral. The increase was expected to be rapid, while the tailing off might begin after the second year, or later. The exact timing of the activity decrease would depend on the particular life histories of the colonizing species.

Iridomyrmex activity at baits in the fire area consistently exceeds that in the control (one-tailed $p = .0078$, Wilcoxon matched-pairs signed-ranks test, Table 1). Within the burned area, there is no monotonic trend in Iridomyrmex activity with time (Kendall's $\tau = .333$, $p > .05$). However, as in the case of species diversity, it appears that Iridomyrmex activity patterns may be characterized by two temporal

phases, the first two years and the second two. During the first 26 months, there is a significant correlation between Iridomyrmex activity and time since fire (Kendall's $\tau = .733$, $p < .05$, Table 3). During the next 27 months, Iridomyrmex activity does not increase with time (Kendall's $\tau = -.200$, $p >> .05$). Iridomyrmex activity is strongly correlated with temperature (Kendall's $\tau = .600$, $p < .01$), and the effect of temperature accounts for over one-third of the correlation between activity and time after fire during the first 26 months (Kendall's partial $\tau = .423$). During this same time period, the correlation between Iridomyrmex activity and temperature (Kendall's $\tau = .733$, $p < .05$) is actually greater when the effect of time since fire is partialled out (Kendall's partial $\tau = .846$).

The strong correlation between Iridomyrmex activity and temperature may be "partialled out" in yet another way. In Figure 4, activity through time is graphed separately for May and July samples. In both cases, the trend through time is the same. There is first a rise in activity and then a drop. This is consistent with the predictions based on social structure. Activity during July is consistently greater than that during May. Iridomyrmex activity is elevated during warm periods within a day also. Iridomyrmex is more active at midday on hot days, when temperatures are highest, than are either of the other two common dolichoderines, Conomyrma bicolor or Conomyrma insana (Figure 5).

The thermophilic nature of Iridomyrmex activity is also reflected in its relatively high critical thermal maximum (CTM). The CTM of Iridomyrmex pruinosum is slightly over 49° C; while that of C. bicolor

Table 3. *Iridomyrmex pruinosum* activity at baits in the burned area related to time since fire and maximum air temperature on the sampling day. Significant correlations are indicated by an asterisk (*).

Comparison	Time Period	Correlated?	Kendall's τ	p
Activity and time (in months) since fire	Whole period (53 mos.)	NO	.333	>.05
	First 26 mos.	YES	.733	<.05
	Next 27 mos.	NO	-.200	>.05
Activity and temperature (maximum daily air temperature)	Whole period	YES	.600	<.01
	First 26 mos.	YES	.733	<.05
	Next 27 mos.	NO	.200	>.05
Activity and time with temperature partialled out	Whole period	NO	.075	-
	First 26 mos.	MAYBE	.423	-
	Next 27 mos.	NO	-.167	-
Activity and temperature with time partialled out	Whole period	MAYBE	.533	-
	First 26 mos.	YES	.846	-
	Next 27 mos.	NO	.167	-

- No probability values available.

Figure 4. Iridomyrmex pruinosum activity following chaparral fire. For three May samples and three July samples, I. pruinosum activity is plotted against time. The time of the fire is indicated. For each month, there is a trend for activity to first increase and then decrease. Activity is measured as percent occurrence at honey-water baits. The maximum possible activity is 100% when I. pruinosum was present at all baits along the transect through the recently burned chaparral.

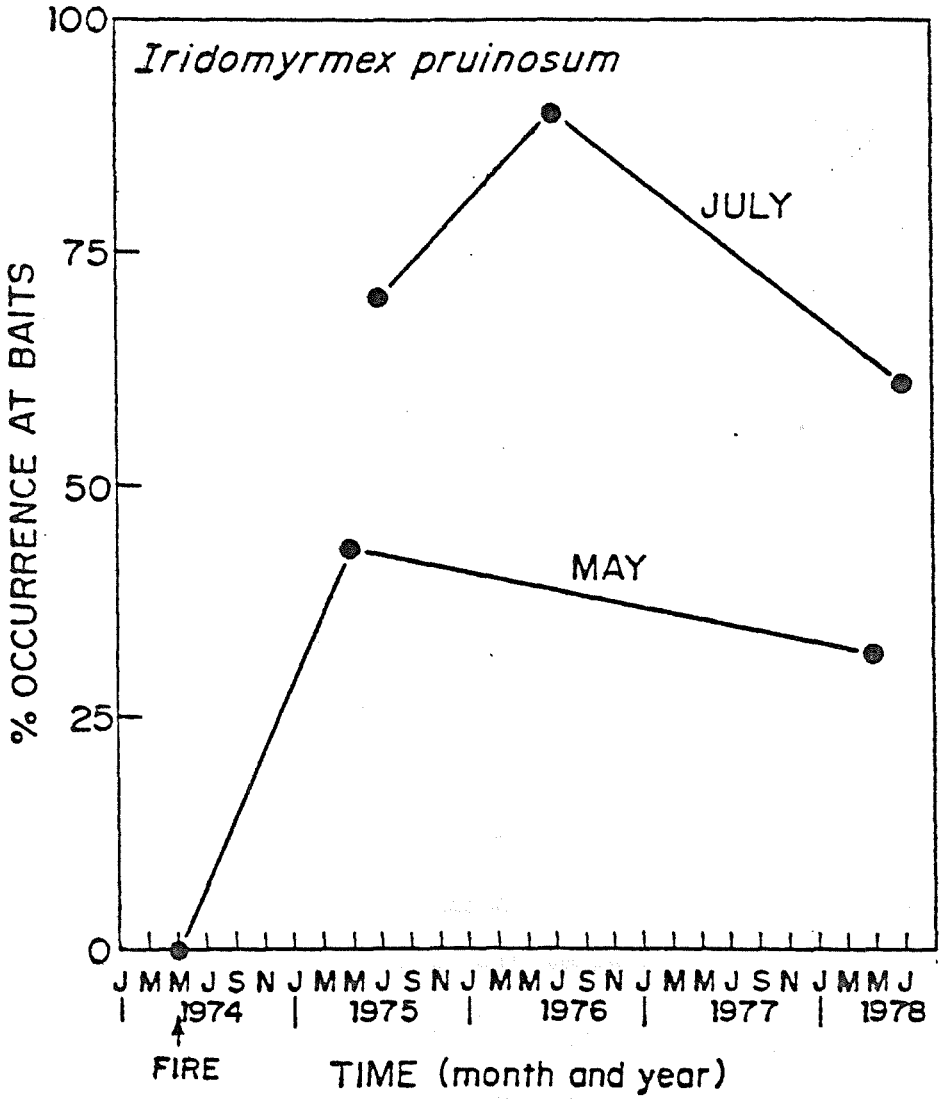
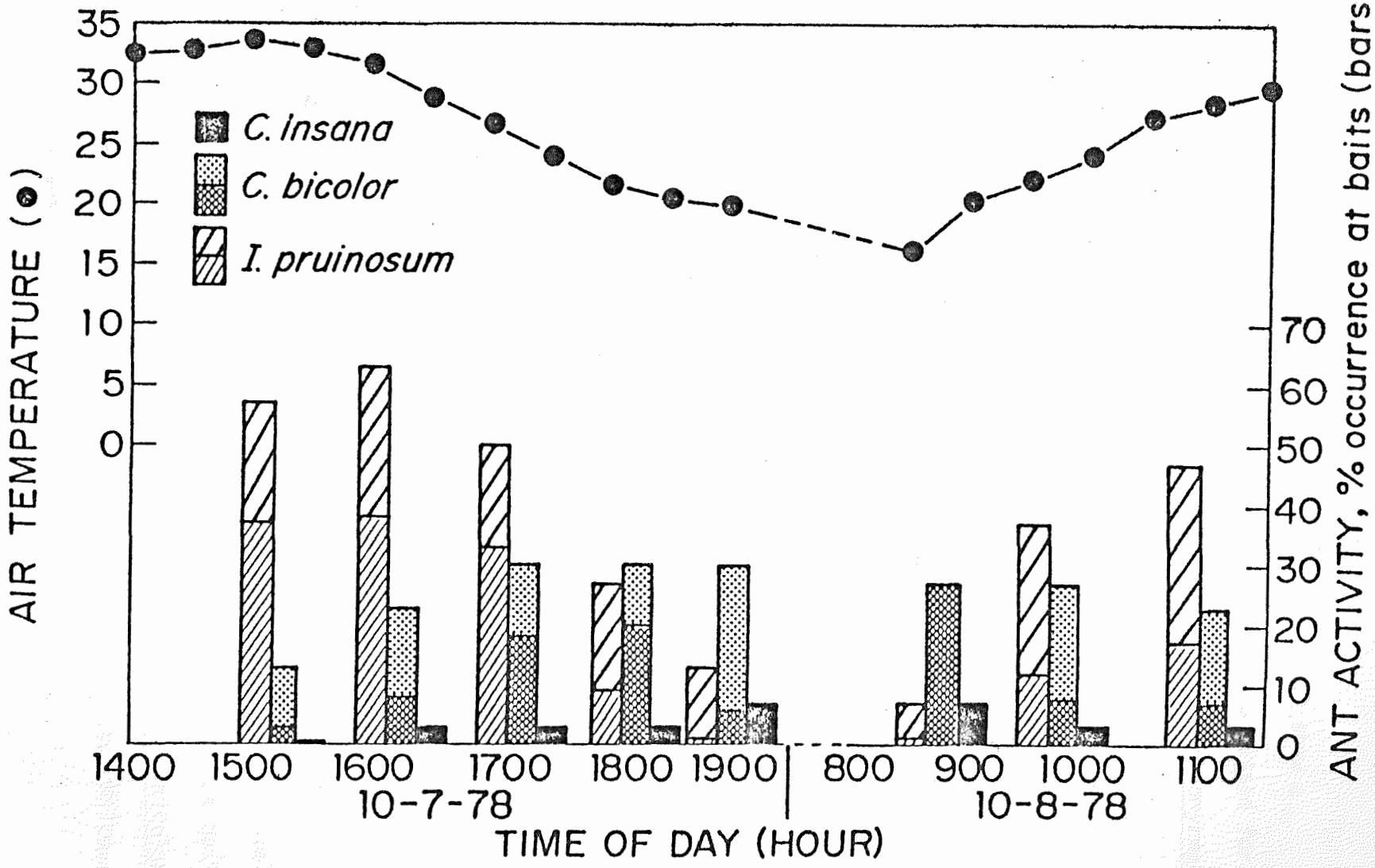

Iridomyrmex pruinosum
JULY
MAY
100
75
50
25
0
% OCCURRENCE AT BAITS
J M M J S N J M M J S N J M M J S N J M M J S N J M M J
1974
1975
1976
1977
1978
FIRE
TIME (month and year)

Figure 5. Diurnal activity of three species of ants. Activity levels of three species of dolichoderine ants, Iridomyrmex pruinosum, Conomyrma bicolor, and Conomyrma insana are plotted against time of day as is the air temperature. Air temperature was recorded in the sun at 5 cm above ground; the bulb of the thermometer was shaded to prevent direct heating by the sun. Ant activity (entire bar for each species) represents the percent occurrence of the species at baits at the given hour of the day in question. Because the number of ants at a given bait was highly variable, activities at baits for Iridomyrmex and C. bicolor were weighted by the mean number of ants of each species at visited baits during the specific sampling period. For these species, 100 ants was taken to represent 100% of the possible number of ants at a bait. Therefore, if the mean number of C. bicolor at visited baits during the period around 1600 hours was 25, then the activity level was multiplied by 25% to get the weighted activity. Weighted activities for Iridomyrmex and C. bicolor are, thus, always less than unweighted activity, and are represented by the bottom (more densely shaded) portion of each activity bar. Iridomyrmex activity is highest during the hottest period of the day, when air temperature is above 25° C. Conomyrma bicolor is most active when air temperature is below 25° C. C. insana activity is highest at air temperatures at or below 20° C. During midday, soil temperatures in the sun were about 10° C greater than air temperatures. In the early and late part of the day, there was little difference between soil and air temperatures. The difference between soil and air temperatures was greater in the afternoon as the air was cooling than in the morning when it was warming.


AIR TEMPERATURE (●)
35
30
25
20
15
10
5
0
C. insana
C. bicolor
I. pruinosum
70
60
50
40
30
20
10
0
ANT ACTIVITY, % occurrence at baits (bars)
1400
1500
1600
1700
1800
1900
800
900
1000
1100
10-7-78
10-8-78
TIME OF DAY (HOUR)

at 46° C. At 0% humidity and 46° C, Iridomyrmex does not show a reaction similar to that of C. bicolor until it has been stressed for twice as long (30 minutes) and at 100% humidity and 46° C, Iridomyrmex shows no ill effects after more than one hour (see Figure 6).

Therefore, Iridomyrmex pruinosum activity shows an activity pattern after fire which is consistent with predictions based on its polygynous social structure. However, the observed increase in activity shortly after fire and subsequent decrease two years later is also explainable (perhaps to a greater extent) by the thermophilic nature of I. pruinosum and its tolerance of extreme high temperatures such as are present in the early post fire period in California chaparral.

Other Dolichoderine Activity

Based on the predictions of the social structure model, we might expect that the two other polygynous dolichoderine species, Conomyrma bicolor and Tapinoma sessile would be rapid post-fire colonizers like Iridomyrmex pruinosum and that the two monogynous dolichoderines, C. insana and Liometopum occidentale would not.

Conomyrma bicolor is among the four most common species at baits in both control and burned areas, and accounts for 10-15% of the total activity at baits along each transect. The percent occurrence at baits for seven paired samples shows there to be no difference in C. bicolor activity between control and burned areas (one-tailed $p > .05$, Wilcoxon matched-pairs signed-ranks test, Table 4).

Figure 6. CTM data for two ant species. Critical thermal maximum behavior for Conomyrma bicolor and Iridomyrmex pruinosum at a series of temperatures is illustrated. At each temperature (up to the maximum) four ants were tested under dry conditions (0% humidity) and four under humid conditions (100% humidity). The time when 50% of the ants showed a loss of the righting response (LRR) under each of these temperature and humidity regimes is indicated.

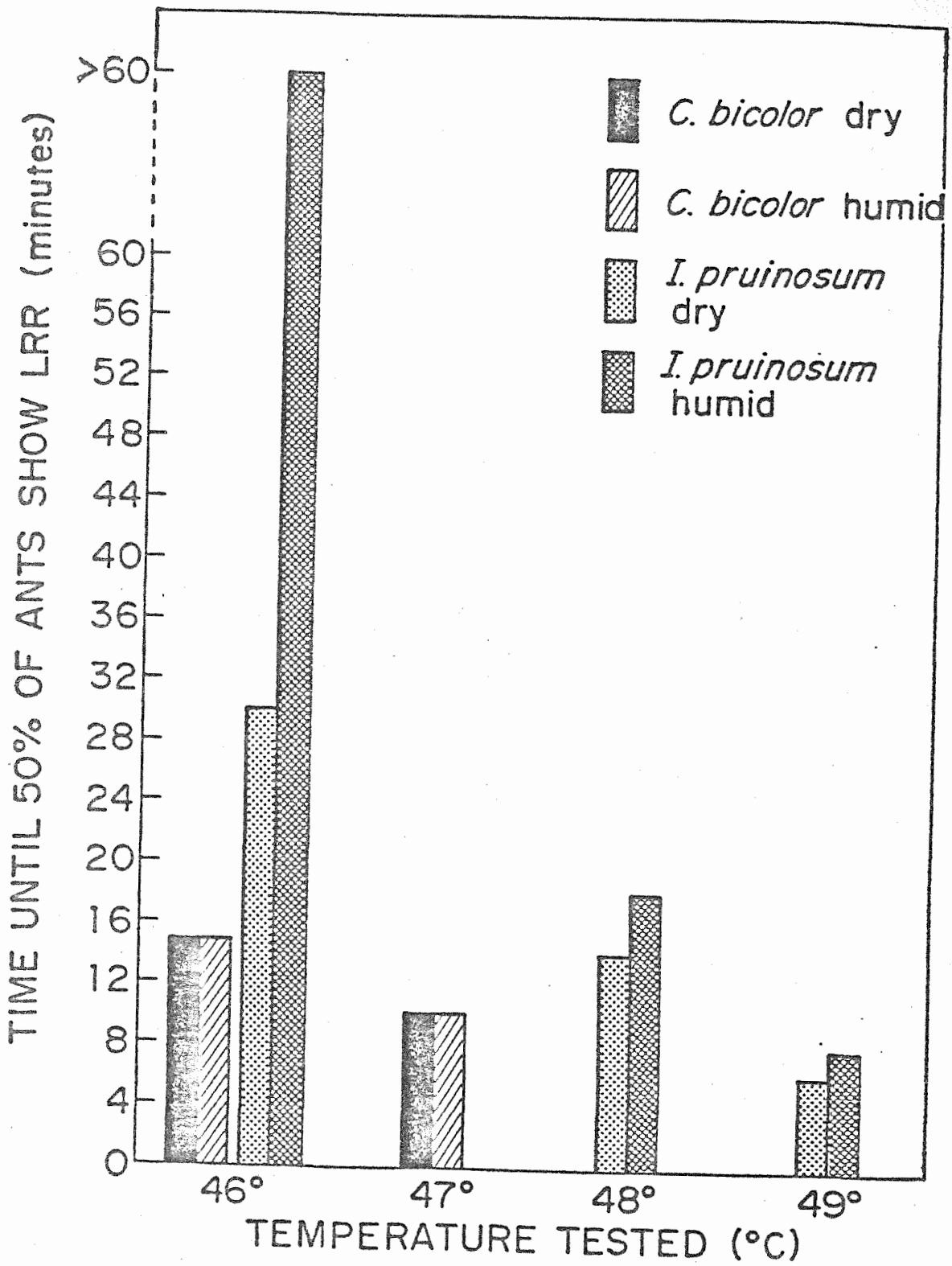

TIME UNTIL 50% OF ANTS SHOW LRR (minutes)
>60
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
C. bicolor dry
C. bicolor humid
I. pruinosum dry
I. pruinosum humid
46°
47°
48°
49°
TEMPERATURE TESTED (°C)

Table 4. Paired comparisons of activities in burned (b) and control (c) areas for Conomyrma bicolor, C. insana, Tapinoma sessile, Liometopum occidentale, Camponotus dumetorum, Formica moki, and Monomorium minimum. Activity is expressed as percent occurrence at baits. Two-tailed probabilities given by Wilcoxon matched-pairs signed-ranks test are given, with significant values indicated by an asterisk (*).

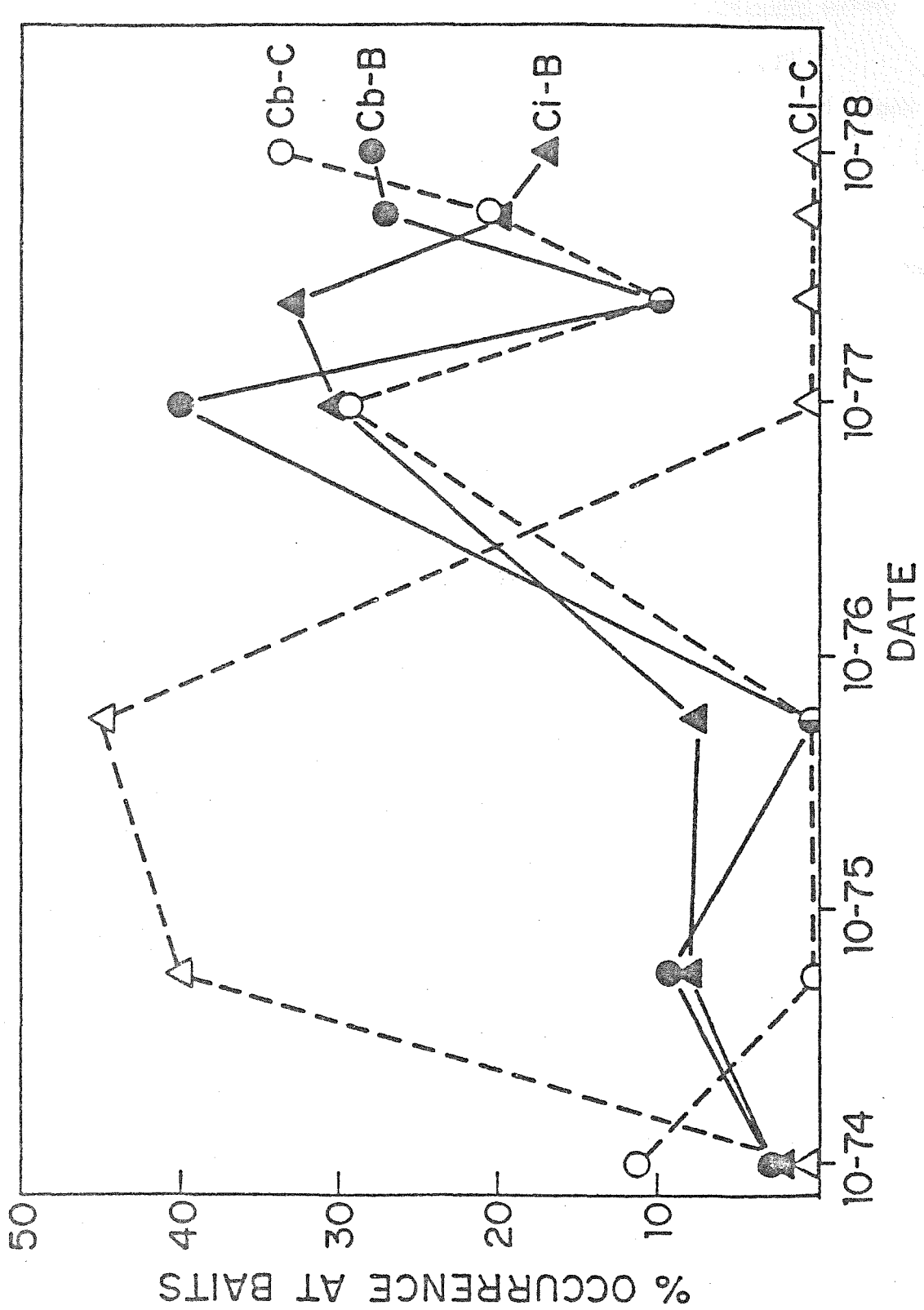
Species	Site	10-74	7-75	7-76	10-77	5-78	7-78	10-78	p
Conomyrma bicolor	b	3	9	0	40	10	27	30	>.05
	c	11	0	0	30	10	20	36	
Conomyrma insana	b	3	8	8	30	33	20	17	>.05
	c	0	40	45	0	0	0	0	
Tapinoma sessile	b	0	4	0	0	10	3	0	<.05*
	c	0	40	45	0	0	0	0	
Liometopum occidentale	b	0	0	0	15	20	10	7	-
	c	0	0	0	0	0	0	0	
Camponotus dumetorum	b	50	40	18	55	70	30	37	<.05*
and Formica moki	c	0	20	15	10	10	0	0	
Monomorium minimum	b	13	2	0	0	0	3	0	<.05*
	c	22	10	15	20	10	32	28	

Tapinoma sessile is common only in the control area, where it accounts for 13% of the activity. It is occasionally found in the burned area and accounts there for only 2% of total activity. The percent occurrence at baits in the two areas is significantly different and in the direction opposite to that predicted on the basis of social structure (two-tailed p=.0312, Wilcoxon matched-pairs signed-ranks test, Table 4).

Conomyrma insana, like C. bicolor, is consistently one of the most abundant species and accounts for 10% of the total ant activity at baits in both burned and control areas. Again, there is no significant difference in the percent occurrence at baits between the two areas (Table 4). There is an interesting contrast to be made between the behaviors of these two species, however, if one looks at the temporal patterns (Figure 7). The activity levels in Conomyrma bicolor vary similarly through time in burned and control areas (Kendall's rank correlation coefficients, burn: $\tau = .524$, $p = .068$, control: $\tau = .390$, $p = .15$). The trend, although erratic, is similar in both areas and may reflect response to weather. By contrast, activity levels in Conomyrma insana show no similarities in the two areas. There is a non-significant trend for C. insana activity in the burned area to increase with time since fire (Kendall's $\tau = .429$, $p = .119$) while the overall trend is the opposite in the control (Kendall's $\tau = -.461$, $p = .191$).

Liometopum occidentale was never recorded from the control area and was not recorded on the burn transect until July, 1976 (26 months after the fire). Since then it has comprised a fairly consistent part

Figure 7. Temporal patterns of activity of two species of Conomyrma in burned and unburned chaparral. Conomyrma bicolor (Cb) is indicated by circles (o) and Conomyrma insana (Ci) by triangles (Δ). Solid lines and symbols are for the burned (B) chaparral and dashed lines are for the control, unburned chaparral (C).


Cb-C
Cb-B
Ci-B
Cl-C
50
40
30
20
10
% OCCURRENCE AT BAITS
10-74
10-75
10-76
10-77
10-78
DATE

(7%) of the total ant activity. One large, active Liometopum colony was observed a year earlier along the burn edge. Liometopum's relatively late occurrence in the burn is consistent with predictions based on social structure.

Among the dolichoderines studied, there is no consistent relationship between social structure and success following fire. The polygynous species, Iridomyrmex pruinosum, and the monogynous species, Liometopum occidentale, behave as expected. A second monogynous species, Conomyrma insana, shows a slight trend in the direction predicted; while a second polygynous species, C. bicolor, shows no difference pre- and post-fire. A third polygynous species, Tapinoma sessile behaves in opposition to predictions based on social structure.

Activity of Non-dolichoderines

Two monogynous (R. R. Snelling, pers. comm.) formicine species, Formica moki and Camponotus dumetorum were regular visitors to the transects. Both are frequently found nesting under rocks and were the two species found most commonly in the burned area immediately following the fire. Together they accounted for 29% of the activity in the burned area and 7% of the activity in the control area. Their percent occurrence at baits throughout the study is significantly different in the two areas and in the direction opposite to that predicted by social structure (two-tailed $p = .016$, Wilcoxon matched-pairs signed-ranks test, Table 4).

One other species, Monomorium minimum, accounts for a large part of the activity in the control area (21%) but is almost lacking (1%)

from the burn transect. The difference is consistent and significant (two-tailed $p < .02$, Wilcoxon matched-pairs signed-ranks test). Colonies of Monomorium may contain several queens (Cole, 1934; Wheeler and Wheeler, 1973) suggesting that polygyny is not uncommon and that reproduction by colony fission may occur. A closely related congener, Monorium pharaonis is a highly polygynous pest species which has abandoned mating flights and reproduces solely by budding (Wilson, 1971). If Monomorium minimum is able to reproduce by budding, we would expect it to be an early colonist following fire. This is obviously not the case.

None of the non-dolichoderine species studied behave as predicted on the basis of social structure. In fact, all three species show significant trends opposite to those predicted; the monogynous species are more common following chaparral fire and the polygynous species is less common.

DISCUSSION

Overall activity of the ant species studied was greater in the burned chaparral than in the unburned control. In this sense, these ants are similar to chaparral plants and predatory vertebrates; while being dissimilar to most soil mesofauna, small mammals, and brush-dwelling birds (Lawrence, 1965). Soil mesofauna in Australian jarrah and karri forests shows both reduced density and species diversity following fire. This is probably the result, initially, of deaths attributable directly to fire. Subsequent reproduction and recruitment are probably diminished for some time afterwards because litter, the major food source for these decomposer organisms, is greatly reduced by fire. Small mammals and brush-dwelling birds, by contrast, do not appear to suffer directly from fire . Lawrence (1966) attributed lowered numbers of these groups following fire to increased apparency and, consequently, predation. That decreased food availability was not the indirect cause, at least for birds, is suggested by the fact that the total densities of nesting birds increased, with increases in grassland and oak-woodland species more than compensating for decreases in brush-dwelling species. Dolichoderine ants suffer very little from predation (Hunt, 1973) and, due to their burrowing habits, ground nesting species should not suffer significant direct fire losses. Increases in their overall activity are, thus, probably due to increased availability of food after fire. That total activity continues to increase for a time after the fire, as does vegetation, supports this idea. The finding that total activity

and Iridomyrmex activity are correlated within the burn and control sites taken together, but not within either site alone, suggests that Iridomyrmex activity is a major component of the differences between these areas.

Since it is the most common species at baits in both burned (40% of total activity) and unburned (21% of total) areas, it is interesting to examine the activity patterns of Iridomyrmex pruinosum in depth. For the first two years, Iridomyrmex activity increased with time since fire. Since then, it has tended to decrease slowly. This is consistent with expectations for an early colonist which comes into an area, expands rapidly, and then moves on. Species diversity in the burned area shows the same two-phase pattern as Iridomyrmex activity. During the first two years, Iridomyrmex activity increases and species diversity decreases. In part, this is a direct effect of the increased abundance of Iridomyrmex as can be seen from the similar two-phase species evenness pattern within the burn. However, when the role of Iridomyrmex is statistically partialled out of the species diversity and time relationship, there is still a very strong negative correlation during the first two years post-fire. During the second two year period, species diversity is at its highest and Iridomyrmex activity has begun to decrease. Species evenness is relatively high during this period, not only due to the decrease in Iridomyrmex activity but also due to increases in the activities of several other previously less common species, such as Conomyrma bicolor and C. insana.

Iridomyrmex was not evident in the burned area ten days after the fire (May, 1974). It was observed one month after the fire (June, 1974)

and was common four months later (October, 1974). Even if Iridomyrmex had swarmed in the interim, five months is too short a time for the development of large numbers of good-sized colonies. The increased activity must reflect either increased activity and growth of surviving colonies or movement into the area of new colonies. The latter would be greatly facilitated by the polygynous nature of many Iridomyrmex colonies.

Iridomyrmex is also extremely thermophilic and is able to tolerate extreme heat and aridity better than Conomyrma bicolor, itself one of the most heat tolerant species in the area (Wheeler and Wheeler, 1973). Christensen and Muller (1975) noted that in cleared southern California chaparral, summer soil temperatures regularly exceeded 54° C. In contrast, soil temperatures under shrubs never exceeded 50° C and averaged 42° C. The ability of Iridomyrmex to forage at higher temperatures than other ant species may allow it to forage for a longer time during the day and over greater distances during hot periods. For a scavenging species, this may provide a critical advantage. As the amount of cover increases in the burned area, Iridomyrmex should be at less of an advantage over less thermophilic species. In addition, if the added food supply increases the carrying capacity of the habitat for ants, colonies of monogynous species would only begin to be large enough to detect by the second year after fire. These monogynous species would add to the total diversity of species as the dominance of Iridomyrmex diminished.

The failure of the other polygynous species to predominate following fire suggests that polygyny is not all that is required for

successful colonization. The relatively high levels of Conomyrma bicolor, Tapinoma sessile, and Monomorium minimum activity in the control area suggests that there is an adequate source population for colonization of the nearby burned area. The common occurrence of Conomyrma bicolor in local deserts (Wheeler and Wheeler, 1973), where cover and insolation are comparable to that in the burn, suggests that temperature should not be limiting. One possible explanation for the lack of Conomyrma bicolor in the burn shortly after the fire is that colony reproduction by budding may be quite local. Relatively long distance colonization may depend on new reproductives. The similarity between burned and unburned areas in the pattern of C. bicolor activity may be a coincidence and may be the consequence of two independent fire related events. Most of the colonies that forage in the control area nest along a fire road that was heavily disturbed during fire-fighting. The increase in activity with time in the control may then be due to recovery of colonies which were damaged by bulldozers. The parallel increase in C. bicolor activity with time in the burned area may reflect colonization by alates following fire disturbance and opening of the habitat.

Although neither Monomorium nor Tapinoma were studied in depth, notes from the literature suggest that they are both less thermophilic and more mesic than either Iridomyrmex pruinosum or Conomyrma bicolor (Cole, 1942; Wheeler and Wheeler, 1973). In addition to their apparent preference for more mesic habitats, Tapinoma sessile and Monomorium minimum may be more prevalent in old chaparral than in recent burns because of predation effects. Many authors have pointed out the

increased apparency of prey in recent burns. Although all dolichoderine ants are relatively unpalatable, Tapinoma sessile was much more commonly eaten by the six species of lizards analyzed by Hunt (1973) than was any other dolichoderine species. Monomorium minimum was not abundant enough in Hunt's study area to show up in the gut of any of the lizards sampled. However, in feeding trials with captive horned lizards, Phrynosoma coronatum, a species that is a specialized predator of ants, Hunt found that all three species of myrmicine ants offered were eaten, while the three species of dolichoderine ants offered were rejected.

The increased apparency of ants in the simplified post-fire habitat would be compounded by increased densities of lizards which are potential ant predators following fire (Lillywhite and North, 1974; Lillywhite, 1977). For example, one such species, Sceloporus occidentalis, has a diet comprised of from 66% to 84% ants (Fuentes, 1976).

Of the monogynous species studied, Liometopum occidentale behaves most as we might expect based on social structure. It is a latecomer to the burned area and has been steadily present since its arrival. Conomyrma insana is present early on, but becomes more active after the second post-fire year. Early records may be due to colonies which persisted through the fire, while the more recent activity upsurge is proabably due to colonization. Conomyrma bicolor, it will be recalled, showed essentially the same pattern.

Two of the monogynous species, Formica moki and Camponotus dumetorum, are interesting in that they behave opposite to the

predictions for monogynous species. They are more common in the burned area than the control. Recall that these were also the two most abundant species immediately after the fire (May and June, 1974). They both tend to nest under rocks and, presumably, were able to avoid displacement by fire. Many colonies of other soil nesting species may not have been killed directly, but may have been forced to relocate after fire. For a combination of reasons, then, these two species entered the post-fire period with well established colonies and were able to reproduce into the area and colonize new patches following fire. Camponotus and Formica are relatively large bodied ants whose colonies are relatively deep nesting and slow growing. Iridomyrmex, on the other hand, is a small bodied ant with short-lived females, shallow nests and rapid colony growth. Iridomyrmex is successful following fire by virtue of its ability to invade, whereas Camponotus and Formica are successful following fire by virtue of "holding on" through the fire.

Therefore, social structure alone is neither a necessary nor sufficient pre-adaptation to the post-fire success of an ant species. The tolerance of the species to the environmental extremes to which it may be exposed following fire is an important additional consideration, as are the nesting habits of the species and its susceptibility to predation.

CONCLUSIONS

This study has shown the following:

1. Total ant activity in the first four and one-half years following a chaparral fire was greater than in a comparable unburned area.
2. Species diversity of ants in recently burned chaparral decreased for the first two post-fire years and then increased. During the period of decrease, species evenness was low when compared with that in the control area.
3. Total activity differences between burned and unburned areas are largely accounted for by high levels of Iridomyrmex pruinosum activity. Iridomyrmex pruinosum is highly polygynous and extremely heat tolerant, two characteristics which make it well suited to post-fire chaparral habitats. Iridomyrmex activity levels are relatively high during the first two years following fire and then decrease to levels seen in unburned chaparral.
4. Three other polygynous species, Conomyrma bicolor, Tapinoma sessile, and Monomorium minimum show post-fire behavior which differs from that of I. pruinosum. C. bicolor shows no difference between burned and unburned areas while T. sessile and M. minimum show higher activity in the unburned areas. It is hypothesized that Tapinoma and Monomorium are relatively unsuccessful in the post-fire habitat because of their preference for mesic habitats and because of their susceptibility to the increased predation dangers following chaparral fire.

5. Of the monogynous species studied, two are relatively slow to enter the post-fire habitat (C. insana and Liometopum occidentale) while two are quick to recover following fire (Formica moki and Camponotus dumetorum). It is hypothesized that the two rapid recoverers are able to do so by virtue of having better withstood the direct effects of the fire.
6. There are no simple correlations between social structure and recovery behavior of ants following chaparral fire. While polygyny may provide an advantage early on, it must be accompanied by tolerance of the environmental extremes to which organisms are exposed in recently burned chaparral. In addition to tolerance of environmental extremes and social structure, it is important to examine the nesting habits of a species when evaluating its recovery potential.

LITERATURE CITED

Christensen, N. L. and C. H. Muller, 1975. Effects of fire on factors controlling plant growth in Adenostoma chaparral. Ecol. Monog. 45:29-55.

Cole, A. C., 1934. An annotated list of the ants of the Snake River plains, Idaho (Hymenoptera: Formicidae). Psyche 41:221-227.

Cole, A. C., 1942. The ants of Utah. Am. Midl. Nat. 28:358-388.

Fuentes, E. R., 1976. Ecological convergence of lizard communities in Chile and California. Ecology 57:3-17.

Gillon, D., 1971a. The effect of bush fire on the principal pentatomid bugs of an Ivory Coast savanna. Tall Timbers Fire Ecology Conference, Proc. 11:377-417.

Gillon, D., 1971b. The effect of bush fire on the principal acridid species of an Ivory Coast savanna. Tall Timbers Fire Ecology Conference, Proc. 11:419-471.

Hanes, T. L., 1971. Succession after fire in the chaparral of southern California. Ecol. Monog. 41:27-52.

Hunt, J. H., 1973. Comparative ecology of ant communities in Mediterranean region of California and Chile. Ph.D. Thesis, University of California, Berkeley.

Lawrence, G. E., 1966. Ecology of vertebrate animals in relation to chaparral fire in the Sierra Nevada foothills. Ecology 47:278-291.

Lillywhite, H. B., 1977. Effects of chaparral conversion on small vertebrates in southern California. Biol. Conserv., 11:171-184.

Lillywhite, H. B. and F. North, 1974. Perching behavior of Sceloporus occidentalis in recently burned chaparral. Copeia 1974:256-257.

Main, A. R., 1980. Fire tolerance of heathland animals. In Specht, R. L. (ed.), Heathland and Related Shrublands. Part B. Analytical Studies. Elsevier, Amsterdam.

Preston, F. W., 1962. The canonical distribution of commonness and rarity: Part I. Ecology 43:185-215.

Preston, F. W., 1962. The canonical distribution of commonness and rarity: Part II. Ecology 43:410-432.

Rundel, P. W. and D. J. Parsons, 1980. Nutrient changes in two chaparral shrubs along a fire-induced age gradient. Am. J. Bot. 67:51-58.

Wheeler, G. C. and J. Wheeler, 1973. Ants of Deep Canyon. Publication of Philip L. Boyd Deep Canyon Desert Research Center, University of California, Riverside, 162 pp.

Wheeler, W. M., 1905. The North American ants of the genus Liometopum. Bull. Amer. Mus. Nat. Hist. 21:321-333.

Wilson, E. O., 1961. The nature of the taxon cycle in the Melanesian ant fauna. Am. Nat. 95:169-193.

Wilson, E. O., 1971. The Insect Societies. Harvard Univ. Press, Cambridge, Massachusetts, 548 pp.

CHAPTER 7. Summary and conclusions

This dissertation addresses three major questions about ant social structure. First, how does polygyny bear upon the genetical theory of the evolution of eusociality? Second, what are some of the ecological advantages of polygyny? And third, how is the level of intraspecific genetic variation related to social structure in ants, specifically, and in Hymenoptera, in general?

To examine the bearing of polygyny on the genetical theory of the evolution of eusociality, I first determined which of the three species studied was functionally polygynous and which monogynous (Chapter 3). In the populations studied, Conomyrma bicolor and Iridomyrmex pruinosum colonies were frequently polygynous. Based on genotype frequencies within colonies, it was concluded that polygyny in C. bicolor might be both more common and more widespread geographically. In contrast, Conomyrma insana was found to be functionally monogynous.

The genetical theory of the evolution of eusociality in the Hymenoptera is founded on a haplodiploidy based, kin selection model. This model assumes monogyny, and predicts that polygyny will only occur where population genetic structure is viscous. Analysis of electrophoretic variability in the three species studied by F-statistics and modified genetic distance measures (D) showed that population structure in one of the polygynous species, I. pruinosum, is in fact quite viscous as compared with that of the monogynous species, C. insana. Analysis of colony gene frequencies within subpopulations suggests that colony reproduction by budding may be a factor contributing to the observed viscosity. The second polygynous

species, C. bicolor, is not particularly viscous when compared with its congener, C. insana (Chapter 4).

The viscosity predicted by the haplodiploidy based, kin selection model, and seen in I. pruinosum, as also predicted by a model, outlined in Chapter 4, based on group selection among trait groups. This model is more general than is the kin selection model. Therefore, although the data gathered are, in part, consistent with the genetical theory, they are not uniquely explained by it. The pattern observed in C. bicolor is not clearly explained by either the kin selection or the group selection model.

Two potential ecological advantages of polygyny were examined (Chapters 5 and 6). Laboratory rearing studies showed that in C. bicolor, a frequently polygynous species, polygyny may result in increased early growth rates and colony survival. The population studied appeared to be incapable of independent, claustral colony founding. Instead, queens appeared to require feeding while rearing the first brood. Field observations of incorporation of newly mated females into existing colonies, combined with laboratory rearing data, led to the hypothesis that C. bicolor may exhibit secondary polygyny which may sometimes be accompanied by colony reproduction by budding. It is not known how general this pattern may be among the C. bicolor populations studied. In contrast, the few C. insana colonies reared in the laboratory did not tolerate pleometrosis and appeared capable of claustral, monogynous colony founding.

Field colonization following chaparral fire showed no clear pattern between social structure and post-fire activity. It had been

predicted that polygynous, budding species would be more prevalent immediately following fire than would monogynous, claustral founding species. This appears to be the case for I. pruinosum but not for C. bicolor. Of these two species, I. pruinosum is the more thermophilic and is better able to tolerate the extremely hot temperatures to which species may be exposed shortly after chaparral fire in southern California. The other dolichoderine, formicine, and myrmicine species studied, similarly, fail to show a consistent relationship between social structure and post-fire colonizing ability. Although social structure may be an important component of I. pruinosum's success, it is neither a necessary nor sufficient preadaptation to post-fire success in general. Equally important factors to consider when evaluating the recovery potential of a species after fire are: its tolerance of environmental extremes, its nesting habits, and its vulnerability to predation.

Genetic variability among the Hymenoptera has been found to be low compared with that of other insects that have been studied. The species I studied were no exception. The mean heterozygosity (H) in a total of 16 populations ranged from .035 to .102 with an overall mean of .059. The percent polymorphic loci ranged from 21% to 39% with a mean of 28%.

Two major explanations for the low observed levels of variability have been proposed: haplodiploidy and eusociality. When a large number of advanced eusocial hymenopteran species were compared with solitary species, no difference in level of variability was found, contrary to the prediction based on eusociality as the cause of low

levels of genetic variability among hymenopteran species studied. Surprisingly, however, primitively eusocial species showed lower levels of variability than either those with more complex societies or solitary species. It is hypothesized that these primitively eusocial species may reflect the ancestral condition of contemporary eusocial species, suggesting that eusociality probably evolved among relatively inbred Hymenoptera.

It has also been proposed, in the literature, that polygyny may be a means of increasing genetic variability in eusocial species that live in restricted habitats where effective population size is quite low. Among ant species in this study and those previously reported in the literature, there is no trend for polygynous species to surpass monogynous species in population levels of intraspecific genetic variability.

The findings of this dissertation research only begin to answer the questions posed. The questions asked are important and cannot be answered satisfactorily by data based on relatively small populations of a few species. Repeatedly, the need for taxonomically diverse data has been apparent during this study. It is hoped that these beginnings will open the door to future studies that will refine the answers provided here.

APPENDIX. Gel banding patterns

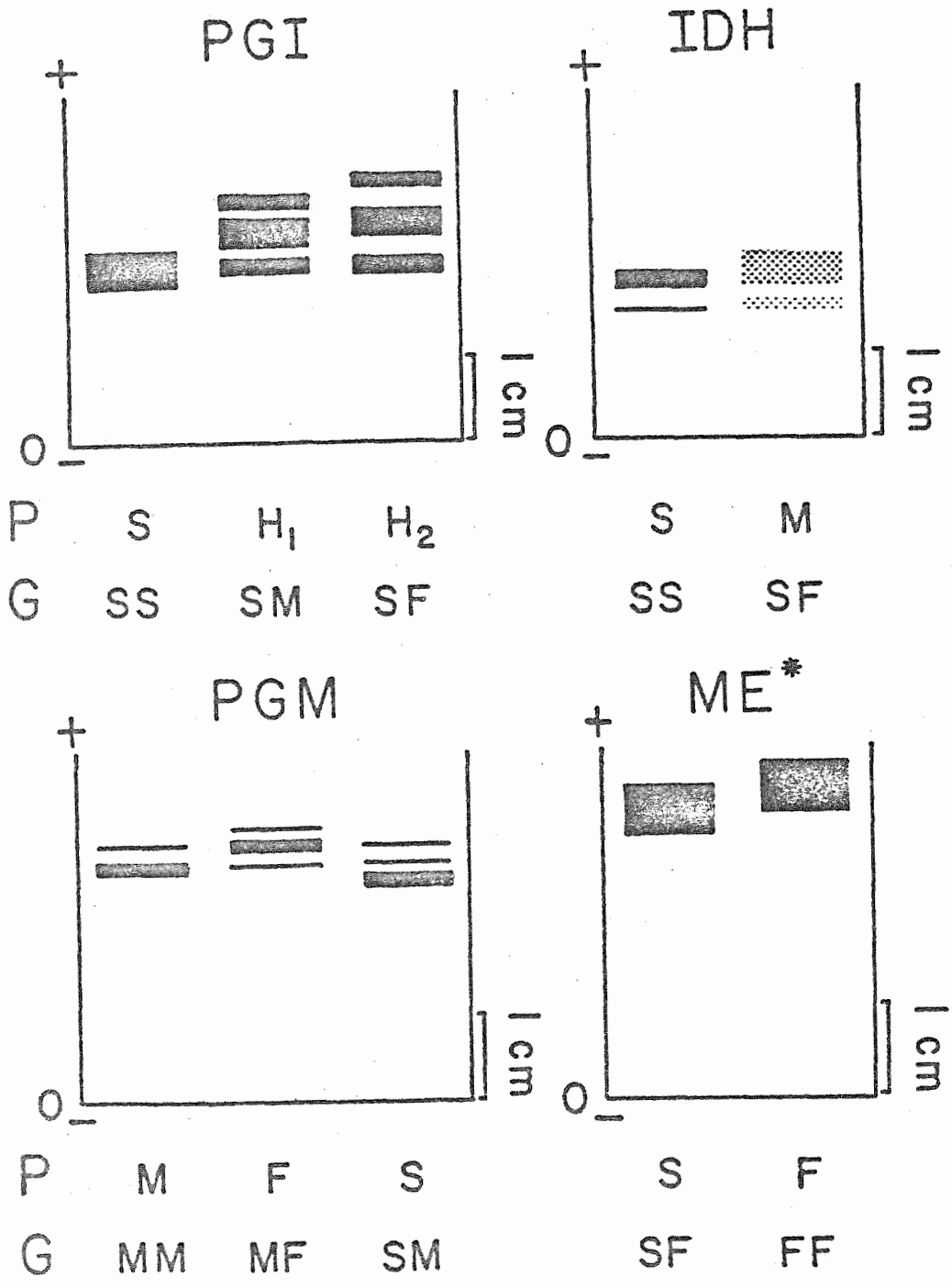
Figures 1-6 illustrate the observed gel banding patterns for allozymes at variable loci in three species of ants, Conomyrma insana, Conomyrma bicolor, and Iridomyrmex pruinosum. Assigning genotypes to phenotypes, sometimes problematical, was done as follows.

For Conomyrma insana (Figures 1 and 2), PGI and MDH had single banded and triple banded forms which could be unambiguously attributed to homozygous and heterozygous genotypes. The simplest explanation is that these enzymes are dimeric with codominant alleles observed for each. Based on experience with IDH in other ant species, the broader, less distinct band pattern observed in some individuals was, similarly, assumed to be the heterozygous form of IDH in C. insana, while individuals with narrower, sharper bands were classified as homozygous.

There were three PGM phenotypes observed in C. insana. All had fast moving sub-bands. Many colonies were monomorphic for the intermediate form, suggesting that it was not a heterozygote. In addition, the "double-banded" nature of the "fast" and "slow" allozymes (actually triple-banded when the fast sub-band is counted) suggested that they were heterozygous forms. No variable colony had all three allozymes; all had the intermediate form and one other. On this basis, it was assumed that the most common allozyme reflected homozygosity, while the two less common allozymes were heterozygous forms. The fact that both presumed heterozygotes were uncommon makes the failure to observe any of the rare homozygous forms unsurprising. The same logic was used in assigning the homozygous genotype to individuals with the common ME allozyme and the heterozygous genotype to the less common allozyme. The failure of ME to show the more typical codominant

Figure 1. Conomyrma insana Allozymes. Zymograms for four loci, PGI (phosphoglucose isomerase), IDH (isocitrate dehydrogenase), PGM (phosphoglucomutase), and ME (malic enzyme) are illustrated. Bands which are stipled were less intense and sharp than were those indicated in solid black. The intensity of stipling indicates the relative band intensity. For each zymogram, the origin is indicated by an "o", the anode by a "+" and the cathode by a "-". Phenotypes (P) are slow (S), medium (M), fast (F), and heterozygous (H_1 and H_2). Phenotypes were considered heterozygous only when they were conspicuously codominant. Genotypes (G) indicate which combinations of slow (S), medium (M), and fast (F) alleles are thought to have contributed to the phenotype. The asterisk (*) following the ME symbol indicates that genotypes for this system were assigned strictly on the basis of rarity and commonness of the phenotypes within colonies (see text); no intermediate form was found.

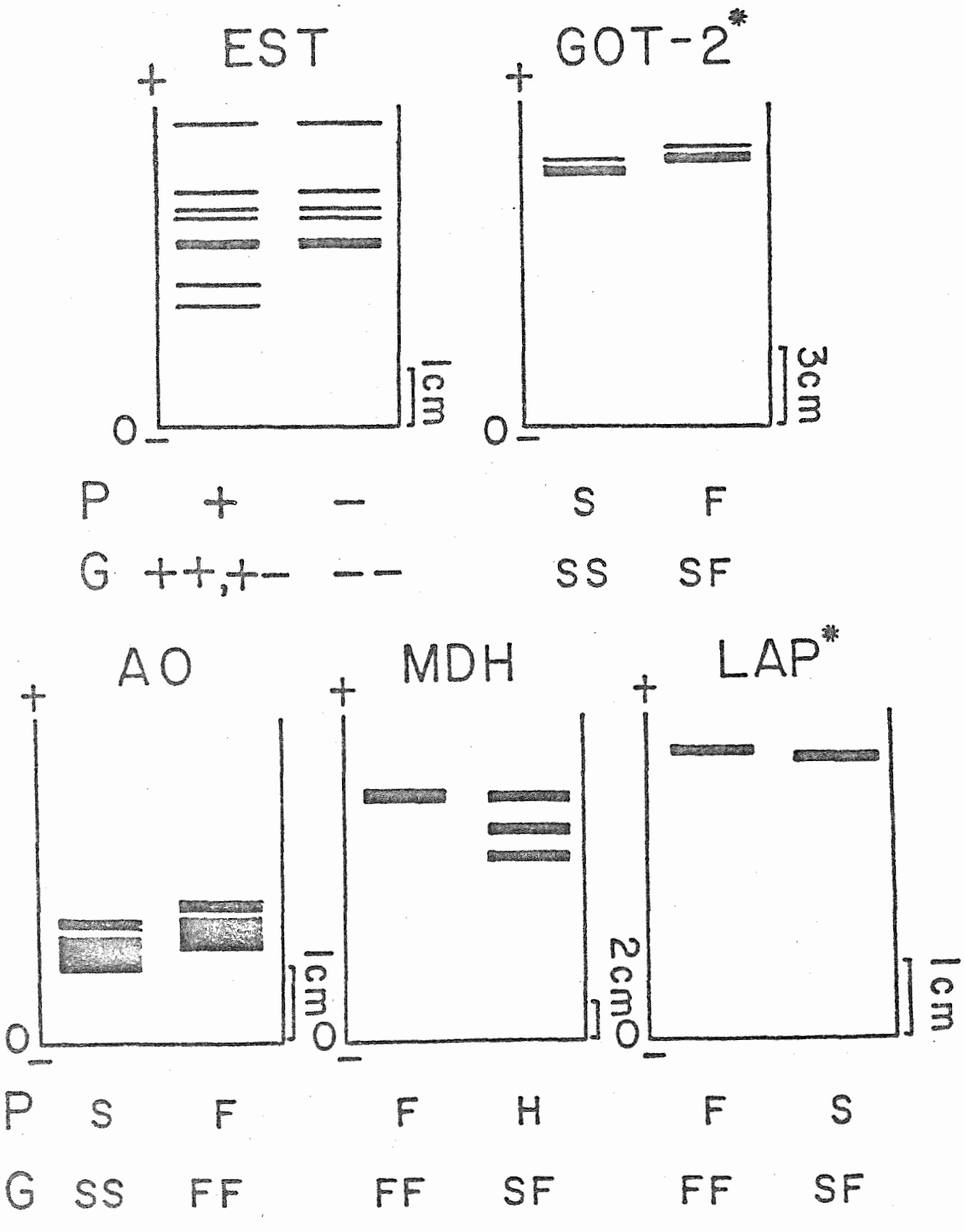
Conomyrma insana Allozymes



P = Phenotype G = Genotype

Figure 2. Conomyrma insana Allozymes (cont.). Zymograms for five loci, EST (esterase), GOT-2 (anodal glutamate-oxaloacetate-transaminase), AO (aldehyde oxidase), MDH (malic dehydrogenase, and LAP (leucine amino peptidase) are illustrated. Zymogram labels, phenotypes, and genotypes are as indicated for Figure 1. In addition, phenotype H is heterozygous codominant. EST phenotypes are indicated as + and - to note presence and absence, respectively, of the two slowest bands. LAP* is as ME* in Figure 1.

Conomyrma insana Allozymes (cont.)



P = Phenotype G = Genotype

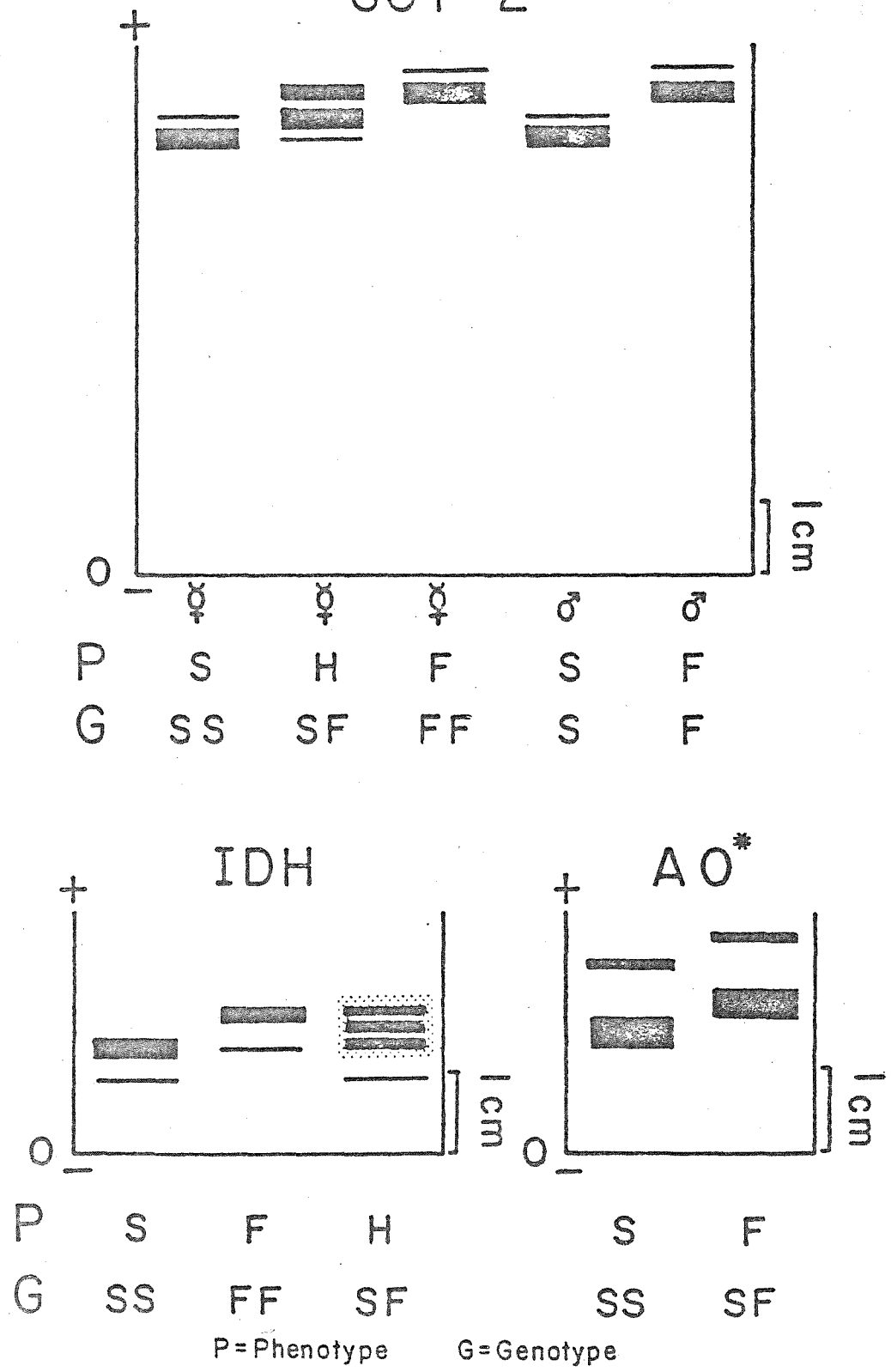
banding pattern seen in PGI, PGM, and MDH, suggests one of the following: (1) the locus exhibits codominance, (2) the genetic variation is at a regulatory locus which affects the product of the structural locus, or (3) the genetic control of the locus is polygenic. Two other loci in C. insana, GOT-2 and LAP, behaved like ME and were interpreted similarly. The pattern at the AO locus was similar, but the distribution of allozymes among colonies differed and suggested a different interpretation. The two allozymes never occurred within one colony and the less common allozyme was only found at two sites where all individuals of all colonies had that allozyme. On the basis of this information, the allozymes were both considered homozygous forms for different alleles.

Finally, EST patterns either lacked both of the two slowest bands or they were both present. It is assumed that the allele for band presence is dominant to the null allele. Therefore, null phenotypes must be homozygotes, while phenotypes with the bands present may be either genotypically heterozygous or homozygous.

In Conomyrma bicolor (Figures 3 and 4), GOT-2, IDH, and PGI showed intermediate codominant forms which were interpreted as heterozygous. The absence of these forms in males for loci examined (GOT-2 and PGI) corroborates this interpretation since males are usually hemizygous. Three loci, AO, EST-6, and LDH, showed no co-dominant intermediate forms. So the more common form was, again, assumed to be homozygous and the less common form heterozygous. If the reverse were true, we would expect to see a third form, the other

Figure 3. Conomyrma bicolor Allozymes. Zymograms for three loci, GOT-2 (anodal glutamate-oxaloacetate-transaminase), IDH (isocitrate dehydrogenase), and AO (aldehyde oxidase) are illustrated. Zymogram labels, phenotypes, and genotypes are as indicated for Figures 1 and 2. For GOT-2, workers are indicated by the symbol ☿, males by ♂.

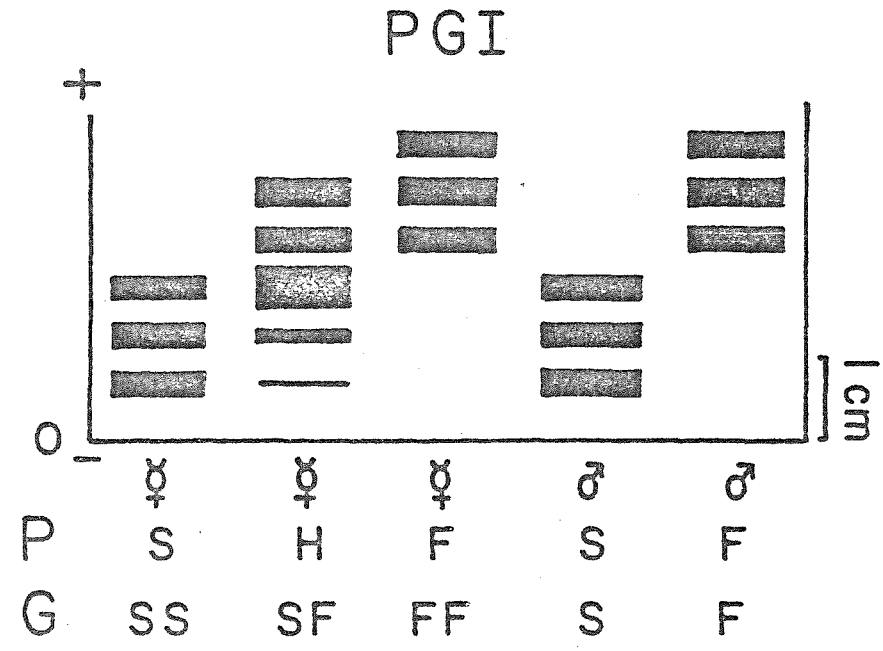
Conomyrma bicolor Allozymes

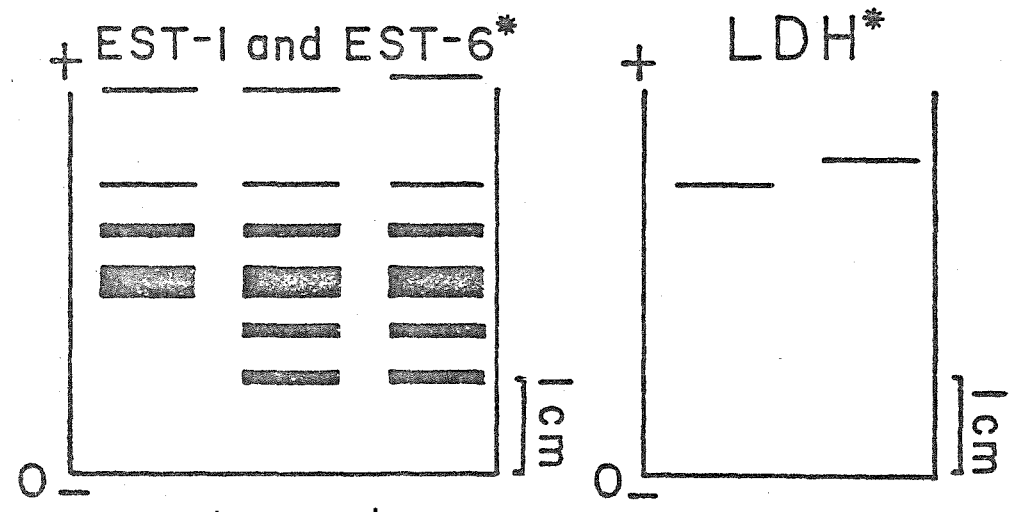


P = Phenotype G = Genotype

Figure 4. Conomyrma bicolor Allozymes (cont.). Zymograms for four loci, PGI (phosphoglucose isomerase), EST-1 (the two most cathodal esterase bands), EST-6 (the most anodal esterase band), and LDH (lactate dehydrogenase) are illustrated. Zymogram labels, phenotypes, and genotypes are as indicated for Figures 1, 2, and 3. For the zymogram where EST-1 and EST-6 are shown, phenotypes and genotypes are given under each individual esterase band pattern with the EST-1 description followed by the EST-6 description. For example, for phenotypes, "EST-1^{-}, 6S" indicates that bands 1 and 2 are absent, while band 6 is slow. An example among the genotypes is " $\begin{matrix}+- \\ \text{or} \\ ++\end{matrix}$, SF ", which indicates that the genotype at the EST-1 locus may be either heterozygous or homozygous for the presence allele. At the EST-6 locus, this individual would be heterozygous (SF).

Conomyrma bicolor Allozymes (cont.)





P	EST-1⁻, 6S	1⁺, 6S	1⁺, 6F	S	F
G	--, SS	+-, SS or ++	+-, SF or ++	SS	SF

P = Phenotype G = Genotype

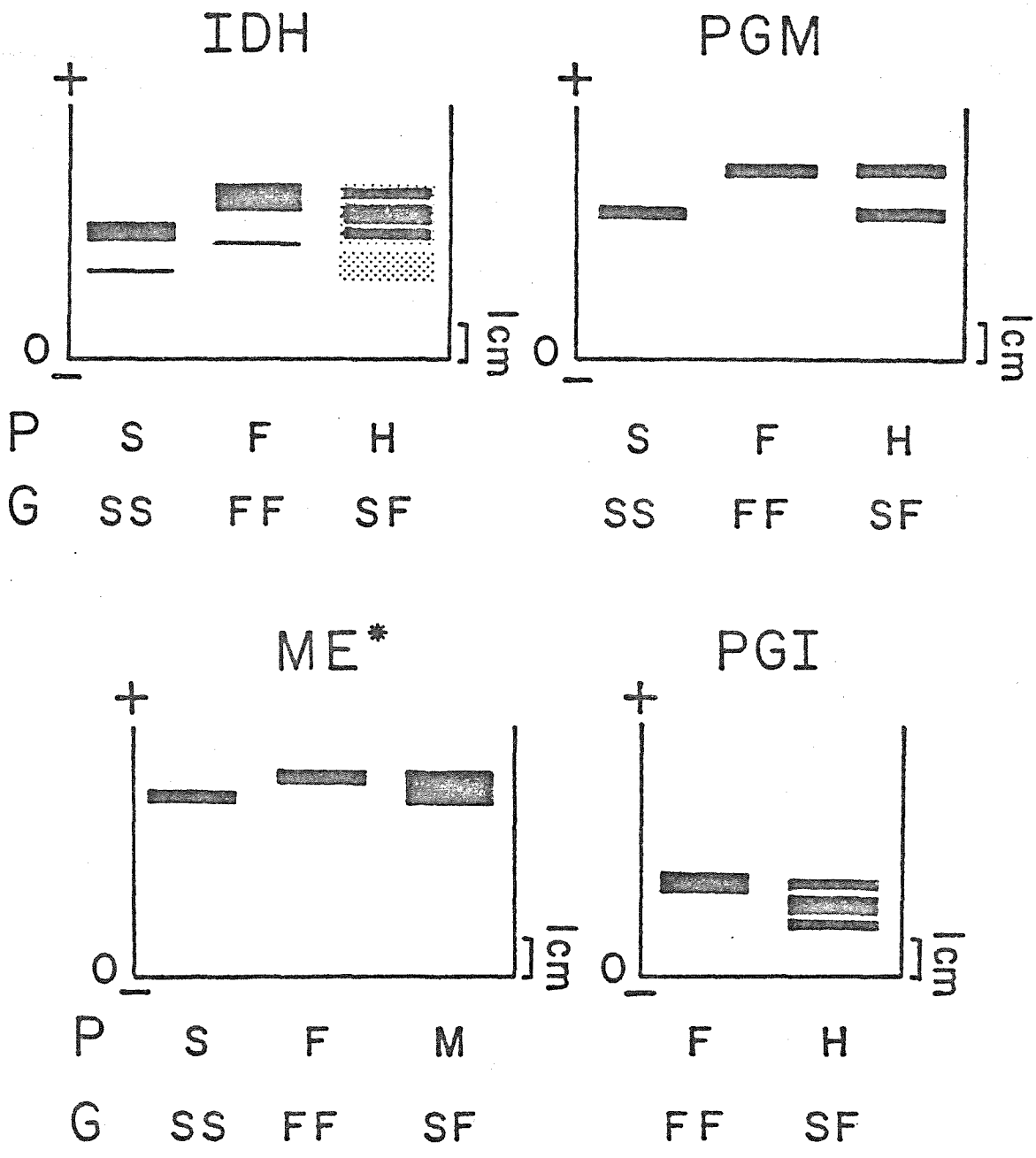
homozygote. As for C. insana, EST-1 in C. bicolor had a null allozymic form which was assumed to be genotypically homozygous.

For Iridomyrmex pruinosum, all systems except ME had intermediate codominant forms which were assumed to be heterozygous. In most cases, both homozygous forms were also present. The indistinctness of ME bands, and the failure ever to see codominance (although there was an intermediate form) made the genotype assignments tentative (Figures 5-6).

All loci (for any species) for which the determination of heterozygotes was unclear (that is, was assumed on the basis of rarity, instead of on the basis of the existence of an intermediate codominant form) or for which there was no unique phenotype corresponding to the heterozygous genotype (that is, for EST loci with null alleles) were excluded in the more conservative estimate of genetic variation (H*) calculated in Chapter 2. Nor were any such loci used in genetic structuring or mating system analyses (Chapter 4). They were, however, included in estimates of percent polymorphic loci (P) and in the less conservative estimate of heterozygosity (H). For more information on H and H* see Chapter 2.

Figure 5. Iridomyrmex pruinosum Allozymes. Zymograms for four loci IDH (isocitrate dehydrogenase), PGM (phosphoglucomutase), ME (malic enzyme), and PGI (phosphoglucose isomerase) are illustrated. Zymogram labels, phenotypes, and genotypes are as indicated for Figures 1-4.

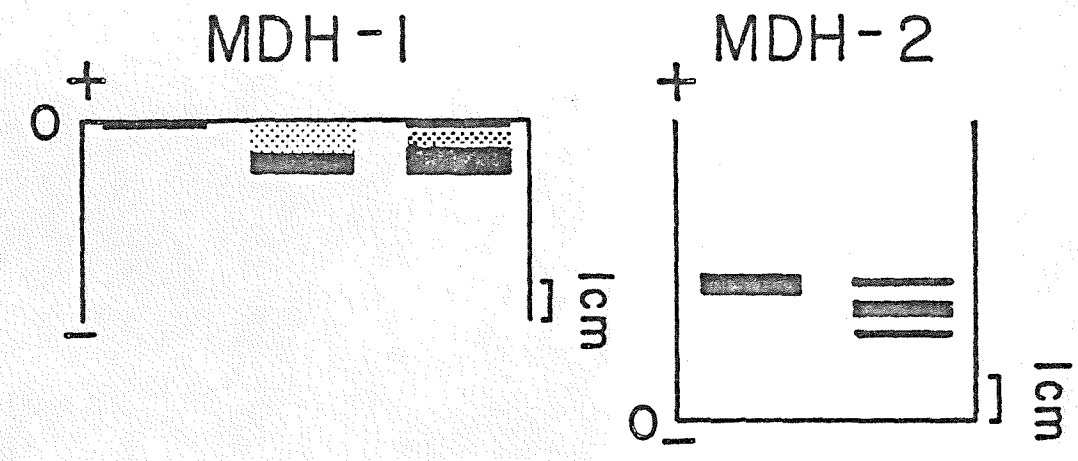
Iridomyrmex pruinosum Allozymes



P = Phenotype G = Genotype

Figure 6. Iridomyrmex pruinosum Allozymes (cont.). Zymograms for two loci, MDH-1 (cathodal malic dehydrogenase) and MDH-2 (anodal MDH) are illustrated. Zymogram lables, phenotypes, and genotypes are as indicated for Figures 1-5.

Iridomyrmex pruinosum Allozymes (cont.)



P = Phenotype G = Genotype