GENETIC STUDIES OF MEAT ANTS

(*IRIDOMYRMEX PURPUREUS*).

by

R. B. Halliday, B.Sc.(Hons.)

Department of Genetics
University of Adelaide.

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SUMMARY

Meat ants (*Iridomyrmex purpureus* Smith) occur in a number of forms or varieties (approximately eight), which differ in colour and nest structure. However, the absence of clear morphological differences between them has resulted in uncertainty about their taxonomic status and evolutionary relationships. Examination of their ranges throughout Australia shows that they fit into an overall distribution pattern which suggests some degree of ecological differentiation. This is also indicated by detailed examination of areas where two or more colour forms are sympatric. One of the major aims of this project was to determine whether any genetic evidence existed which could be used to clarify the relationships between these forms.

One approach to this problem involves the use of allozyme loci detected by gel electrophoresis. Several polymorphic loci have been identified which show substantial differences in frequency, or an almost complete lack of shared alleles, between some combinations of colour forms. In various areas, it is possible to distinguish almost every colour form, on the basis of its allele frequencies at one or more of these loci. This indicates that they are not interbreeding, and that the meat ant group comprises a complex of sibling species.

Some alleles also show considerable geographic variation in frequency within colour forms, in a way which does not appear to be related to any obvious environmental variables. Factors contributing to this variation could include geographic subdivision of the range of some colour forms, and the fact that breeding only occurs in very specific weather conditions, which may only occur over a small area at any one time. Gene flow between geographically distant populations may therefore be restricted. However, it is not certain whether their gene

frequencies have diverged as a result of differing local selection, or by a combination of founder effects and drift.

Estimating gene frequencies in meat ants is more complicated than in other organisms, since the sterile worker caste is usually all that can be collected. For a locus with co-dominant alleles (*e.g.*, *Amylase*), the genotypes of the sexual portion of the population can be inferred from the genotypes of the workers in each nest. For a locus with dominance this is not possible since heterozygotes cannot be recognized. A maximum likelihood procedure has therefore been used to estimate the gene frequencies of recessive alleles, especially in the case of a null allele at the locus *Esterase-1*.

Several polymorphic marker loci have also provided valuable insights into some aspects of meat ants' social behaviour. In particular, genetic differentiation on a very small scale has been detected, apparently caused by the presence of queens of differing genotypes in colonies which are adjacent, but which do not exchange workers across territorial boundaries.

A total of 15 loci were surveyed in four populations to determine the level of genetic variation present. The average heterozygosity per locus was 3.8% which is much lower than the values for *Drosophila*, other insects, and other invertebrates, but comparable to the results commonly found in vertebrates. It is also very similar to the levels observed in a variety of other species of Hymenoptera which have been surveyed. These species are very diverse in habitat, degree of social behaviour, niche width, and life style. A single explanation for their low levels of heterozygosity in terms of environmental heterogeneity or other parameters is therefore unlikely to be found. Haplodiploidy

itself seems to be the only factor which these species have in common, and both "selectionist" and "neutralist" points of view can provide reasons why this might produce low levels of genetic variability.

This multi-locus survey was also used to derive genetic distances between populations, and a dendrogram was constructed showing the relationships of nine populations, including five colour forms. This did not provide a clear indication of their phylogenetic relationships however, since the extent of geographic variation within colour forms was sometimes greater than the differences between them.

Attempts to establish the relationships among the colour forms using karyotypes and morphological data were also made. These did not provide conclusive results, but when added to the ecological, genetic, taxonomic and behavioural information which is now available they contribute further to increasing our understanding of many aspects of the biology of this group of species.

DECLARATION

This thesis contains no material which has been accepted for the award of any degree or diploma in any University and, to the best of my knowledge and belief, contains no material published or written by another person, except where due reference is given.

R. B. Halliday

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CHAPTER 1

INTRODUCTION

Mayr (1942) introduced the term "sibling species" to describe sympatric groups of organisms which are reproductively isolated, but very difficult or impossible to distinguish on morphological grounds. Such species are not qualitatively different from species which are morphologically distinct, but rather they represent the extreme of a continuum, in which the morphological difference between species steadily diminishes. Sibling species are therefore difficult or impossible to recognize if preserved museum material provides the only available information. Their existence may be suspected however, if a single morphological "species" exhibits inconsistencies in behaviour, ecology, or geographic distribution.

The classic example of this phenomenon comes from mosquitoes of the *Anopheles maculipennis* group (reviewed by Mayr, 1970; Coluzzi, (1970). This group consists of six sibling species, the adults of which are morphologically indistinguishable. However, each species shows a characteristic combination of egg morphology, chromosomal rearrangements, habitat preference, feeding behaviour, and efficiency as a vector of malaria. Until all this information was considered together, it was not possible to gain a complete understanding of the epidemiology of malaria, or to design efficient control procedures. The point to be emphasized then, is that the recognition of sibling species depends on the collection of a very wide variety of information from ecology and behaviour, as well as biochemical and cytological evidence if it is available.

The principal objective of the research described in this thesis was to apply this multi-disciplinary approach to a group of Australian ants presently known by the single name *Iridomyrmex purpureus* (Smith).

The question of particular interest was whether these ants should be included under a single species name as they are at the moment, or whether they actually constitute a group of sibling species.

I will show in the next Chapter that this "species" (commonly known as meat ants), occurs in a number of "forms", which differ mainly in colour. Colour is difficult to quantify and describe objectively, so the differences between these forms have not usually been thought sufficient to justify their separation at the level of species. However, the existence of this colour variation, together with variation in nest type, suggests that further information should be sought regarding the relationships between these forms.

One technique which has proved useful in discriminating between species in a wide variety of organisms, is gel electrophoresis of enzymes and other proteins. The value of this approach largely stems from the fact that electrophoretic variation in a protein usually reflects variation in the nucleotide sequence of the gene which codes for it. Although this argument makes a series of assumptions, gel electrophoresis is a convenient method for making the closest possible approach to variation at the level of the genes. It may therefore provide information which cannot be obtained from characteristics whose genetic control is less direct. With this in mind, I have sought to apply gel electrophoresis to the problems of distinguishing between the colour forms of meat ants, and of identifying their evolutionary relationships.

Studies were also undertaken of the geographic distributions of the forms, their ecological and behavioural interactions, karyotypes, morphology, and nest types, so that the widest possible variety of information could be employed in the attempt to establish their status.

Meat ants are now sufficiently well known ecologically that it

is possible to undertake detailed examinations of specific aspects of their behaviour. The second major aim of my research was to identify polymorphic gene loci which would provide markers for this purpose, particularly for examining the genetic relationships between the individuals in specific nests and colonies. This approach has the potential of providing information regarding the number of queens in a nest, patterns of reproductive behaviour, and the relationships between different nests within a population. This information can then be combined with data obtained in other ways, such as the mapping of nests and colonies in the field. The results of this work are presented in Chapter 8.

Meat ants are large, common, and easily collected in large numbers. They also have a well developed system of social behaviour. These factors mean that they are convenient material for approaching the third objective of my research - an extensive isozyme survey, aimed at examining some aspects of population genetics which may be general phenomena in Hymenoptera. In particular, it is of considerable interest to determine whether social behaviour and the presence of haploid males have any effect on the level of heterozygosity found in populations.

A survey of isozyme variation in meat ants, coupled with other aspects of their biology, therefore has the potential of answering specific questions about the behaviour and evolution of this group, as well as having some relevance to more general questions of the genetics of Hymenoptera and the relationships between sibling species.

CHAPTER 2

ECOLOGY AND SYSTEMATICS OF MEAT ANTS

2.1. Taxonomic background

Meat ants are endemic to Australia, and form a distinct group within the large genus *Iridomyrmex*. They differ from most other members of the genus by their large size (7-9 mm.), and by their relatively small eyes, whose length is less than half that of the head. Many authors have noted that meat ants occur in a number of different colour forms, all of which are included under the single name *Iridomyrmex purpureus* (Smith) (*e.g.*, Duncan-Weatherley, 1953; Anonymous, 1956; Greaves, 1971).

Although these forms have been recognized as a result of their colour differences, it has not been possible to find any consistent morphological or structural differences between them, which would allow their elevation to the status of separate species. They have therefore been regarded as various infra-specific categories, including varieties, races and subspecies. The current practise is to describe them by the non-commital term "forms" (Greaves, 1971; Greenslade, 1974b), until a formal revision of the group is completed.

Meat ants formally entered the entomological literature for the first time in 1858, when Smith (1858, page 36) described a female of *"Formica detecta"*, from the Hunter River, New South Wales. Frogatt (1907) claimed that Smith had described a male but this is not correct. Smith did, however, describe a worker of this species in the same paper (Smith, 1858, page 40), under the name *"Formica purpurea"*. His description of this worker as "bright red with a faint purple tint", and its collection locality (Melbourne), make it clear that he was referring to what is now known as the red form of meat ants. This form has therefore come to be known as *purpureus sens. strict.* The listing provided by Dalla Torre (1893) shows that Lowne, in 1865, chose the name *"purpurea"* in preference to *"detecta"*, as he recognized that *detecta* was actually the queen of

purpurea (R. W. Taylor, personal communication). However, Dalla Torre (1893) reverted to the name *detectus*. This usage was then adopted by Forel (1910), Emery (1913), and Viehmeyer (1913, 1925), and was used by many subsequent authors (*e.g.*, Wheeler, 1915; Clark, 1924; Currie and Fyfe 1938). In 1968, R. W. Taylor (in a personal communication to J. A. Hilditch) pointed out that the correct name for meat ants was *Iridomyrmex purpureus*, and in that year, the species name *detectus* (Crozier, 1968) and *purpureus* (Ettershank, 1968) both appeared in the literature. Since then the name *purpureus* has been generally accepted, although some exceptions have still occurred (Wilson, 1971; Beattie, 1972).

Dalla Torre (1893) listed no less than 12 names which had been applied to meat ants to that time and their naming has changed several more times since then. All these changes, however, have dealt with a single colour form (red).

The first description of any colour form other than red was that of Forel (1910), who described the variety *sanguinea* (later known as *sanguineus*). He described specimens of this form from Mackay and Townsville, in Queensland, as being of a clear red colour, with much weaker iridescence than *purpureus*.

Viehmeyer (1913) then described another variety which he called *Iridomyrmex detectus* var. *viridiaeneus* (now commonly referred to as the black form). He described the worker as very dark, with a dark green head and thorax, and with violet reflections on the gaster and legs. Two years later Wheeler (1915) examined metallic green specimens from northwestern South Australia, and correctly identified them as *viridiaeneus*. However, Clark (1938) applied the name *viridiaeneus* to meat ants from Reevesby Island, South Australia, which were "deep metallic blue on the head", from nests which consisted of a "very small hole without a mound". These were probably not true *viridiaeneus*, but another unnamed form,

which is now known as the blue form (Greenslade, 1974b). The inclusion of both blue and black forms under the name *viridiaeneus* led to some misclassification of specimens, and caused confusion in subsequent publications. The term *viridiaeneus* was used to include both blue and black forms by Greenslade (1970) and Greaves (1971), and was incorrectly applied to the blue form by Crozier (1968a), Halliday (1972), and Greenslade and Greenslade (1973). It is now clear that there are two dark coloured forms of meat ants in arid and semi-arid Australia - the dark, metallic blue form, which builds simple single hole nests, and the black form *(viridiaeneus)*, which has a green metallic sheen on a brown to black head, and which is usually found in compound, multi-entrance nests (Greenslade, 1974b).

Another name which has been formally applied to meat ants is *Iridomyrmex detectus* var. *castrae* (Viehmeyer, 1925). This name was applied to ants from Liverpool, New South Wales, which differed from the typical *detectus* by being more heavily built and darker, with a strong blue shine, and which came from a single hole nest with no mound. Collections made by myself and others from Liverpool and surrounding areas have yielded only the typical red form, so the status of *castrae* is not clear. It has not been reported since Viehmeyer's original (1925) description, and the possibility cannot be ruled out that his associate "Mr. Overbeck", had collected from a colony of the red form which for some reason had not built a mound.

It is clear from these early studies that the variation in colour and nest form shown by meat ants has caused considerable confusion in much of the published literature concerning them. Eight colour forms can now be reocgnized, and they are described in the next section.

2.2. Appearance of colour forms

The identification of a meat ant as one of the eight presently recognized colour forms, depends mainly on the colour of the head and trunk. In all forms, the gaster is black and pubescent, and there is some variation in the colour and intensity of its iridescence, even within forms. The colour of the head and trunk is made up of two components - a basic pigment conferring the main background colour, and a superimposed iridescence which imparts a metallic sheen, often of a different colour from the pigment. The background colour can be viewed alone by immersing a specimen in alcohol, which suppresses the iridescence. Dry mounted specimens show that the colour of the metallic sheen depends to some extent on the type of illumination used, with fluorescent tubes, incandescent lights, and sunlight, emphasizing different components of the overall colour. Therefore the identity of a specimen is decided by a combination of all of these components of colour. The differences between the forms are probably seen most clearly in living specimens viewed in sunlight, but it is not always possible to observe them in this way. The description of the colour forms given here have been adapted from Greenslade (unpublished manuscript), as well as previous publications and personal observations, and a full account will be given in forthcoming publications (Greenslade and Halliday, in preparation).

Of the presently recognized colour forms of meat ants, the red form, or *purpureus sens. strict.* (P), has been the most commonly collected and the most intensively studied. The body colour of the workers is red to reddish brown, with the head, mandibles, and antennal scapes lighter than the body. Superimposed on the red background is an iridescence, which varies from pink to blue in daylight, but which tends towards violet in fluorescent light. Living specimens appear red with a blue metallic sheen.

The blue form (B) is uniformly very dark brown to black on the head and body, with the mandibles and antennal scapes often somewhat lighter. The iridescence is invariably of a very strong metallic blue colour, which makes the ants appear very dark. Figure 2.1 shows a red form worker and two blue form workers for comparison of their colours.

In the black form *(viridiaeneus*, V), the body colour is dark brown to black, with the head, mandibles, and antennal scapes lighter brown (Figure 2.2). The iridescence on the head usually appears metallic green in daylight, with some additional violet reflections when viewed under fluorescent light. B and V mainly differ in the colour of this iridescence, and there is rarely any problem in distinguishing them. They also differ in size and behaviour, with B usually somewhat smaller and less aggressive than V.

In South Australia, the small purple form (SP) is also smaller than most of the others, as its name suggests (Figure 2.3). Light coloured specimens of SP have a red body colour similar to that of some P specimens, but SP is usually recognizable by its darker purple colour. The front of the head and the dorsum of the trunk are usually infuscated dark purplish brown. SP is best distinguished from P by the fact that the head (dark reddish purple) is usually darker than the trunk (red-brown), while the reverse is true in P.

The yellow form (Y) varies from yellowish brown to dark brown, with the head, mandibles, and antennal scapes lighter than the body. The iridescence is usually metallic green in daylight, with additional violet reflections. It is therefore sometimes difficult to distinguish from V, but Y usually has a lighter coloured head.

The form *sanguineus* (S) has light red to brick red foreparts, with the mandibles, legs, and petiolar node light to dark brown. The iridescence is very weak, and usually colourless. The gaster of S is heavily

Figure 2.1.

Two members of the blue form of meat ants, and one of the red form, photographed near Eudunda, South Australia.

Figure 2.2. The black form *(viridiaeneus)* of meat ants, photographed on the nest, near Morgan, South Australia.

Figure 2.3. The small purple form of meat ants, photographed on the nest, on Eyre Peninsula, South Australia.

pubescent and usually has a greenish gold iridescence (pink in Western Australia).

The orange form (O) has orange foreparts, generally lighter than those of S, with the mandibles, legs, and petiolar node light to dark brown. The iridescence is colourless, but stronger than that of S. O is distinguished from S by the sparse pubescence and stronger iridescence on the gaster of O.

The dark yellow form (DY) resembles Y, but in DY the trunk is much darker and contrasts more strongly with the head. The trunk and legs are dark purplish brown, and the head is paler, yellowish brown. The iridescence is weak green and violet.

Some of the colour differences between forms are subtle and require careful observation. Difficulties in identification may arise with single specimens, since there is variation in the intensity of colouration within nest populations. Further problems may arise with old, faded museum material. Nevertheless, if fresh material is viewed both dry and in alcohol, and preferably alive in sunlight as well, there is usually little doubt as to its identity.

2.3. Geographic distribution

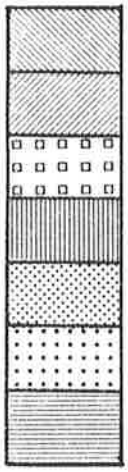
Once the colour forms can be unambiguously recognized, it becomes possible to compile information on their geographic distributions. The distribution map shown by Greaves (1971) includes only three forms, since at that time V and B were not clearly distinguished, P and SP were also combined and other forms had not been recognized. The presently available distribution data for the eight forms referred to here is summarized in Figure 2.4 (based on Greenslade, unpublished results.) The records used in compiling these maps have come from a variety of sources including material from the Australian National Insect Collection, a number of private collections, and recent mapping surveys. The solid areas of shading shown do not necessarily imply the existence of a continuous dense population. Indeed, in many inland areas, little is known of the meat ant fauna. Rather, the areas shown are intended to enclose the regions within which each form is known to occur.

P occupies humid and subhumid areas in eastern and southeastern Australia where the annual rainfall is approximately 350mm. or more (Figure 2.4). It is able to penetrate for considerable distances into drier areas along watercourses, or near human habitations where an artificial water supply allows the growth of large trees (Greaves, 1971). At its western limit on Eyre Peninsula, there is a small area of overlap with SP. The distribution of SP is continuous from there across arid areas into the southern parts of Western Australia.

The form occupying the largest area is V, which is found over most of arid central Australia, and whose distribution includes areas occupied by several other forms. B is restricted to the southern parts of the area occupied by V, and extends beyond this area further south into the semi-arid zone. The form apparently adapted to the most arid areas is O, which seems to occupy two areas separated by the sandy soils of the

Figure 2.4.

Maps of Australia showing the distributions of the eight currently recognized colour forms of Meat Ants.



Small purple form

Red form (*purpureus* sens.strict.)

sanguineus

Blue form

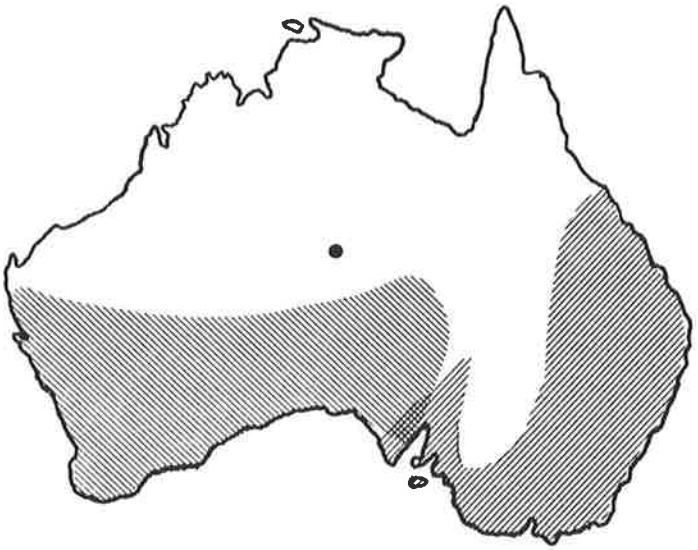
Dark yellow form

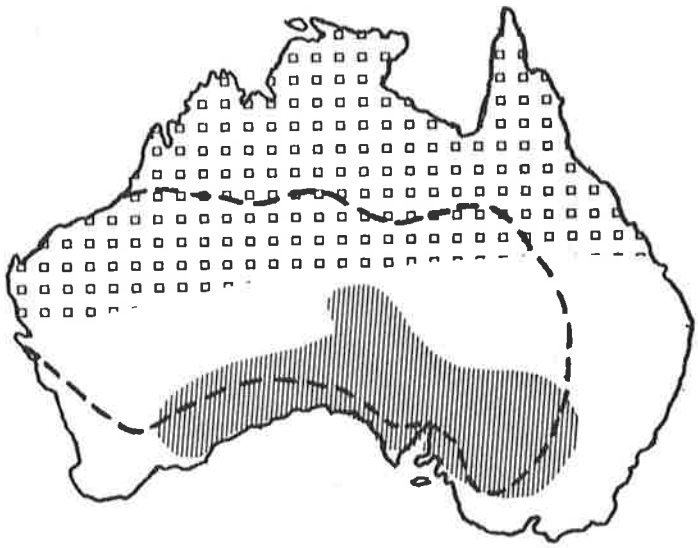
Orange form

Yellow form

Dashed line encloses the distribution of the black form (*viridiaeneus*).

• Isolated record of the red form near Alice Springs





Great Victoria Desert (Figure 2.4). However, parts of the gap between these two areas are rather inaccessible, and further populations could conceivably exist there.

The yellow form (Y) reaches high population densities on sandy soils on Eyre Peninsula, in the region where P and SP occur together. It has also been found in several places east of the Flinders Ranges, and it is well established in the Upper Southeast of South Australia, also on sandy soils (the eastern portion of its range in Figure 2.4). DY also occurs in two disjunct areas, in semi-arid and sub-humid north Queensland.

These overall distribution patterns give preliminary indications of the existence of ecological differences between forms. Y is apparently confined to sandy soils in semi-arid areas, S occupies the tropical north and P apparently needs an assured supply of water, either from rainfall or the soil. Several other forms can exist in areas where water is much less freely available. The maps in Figure 2.4 also serve to identify areas where two or more forms are found together, and it is here that more detailed information concerning the relationships and interactions between them has been obtained.

2.4. Interactions between colour forms

Greenslade(1974c, 1976b) has provided detailed information concerning the distribution of P and B in areas surrounding Adelaide. The most conspicuous feature of their relationship in this area is a sharp boundary, of the order of 200km. in length, where the two forms meet. This boundary corresponds with a zone of very steep environmental gradients along the eastern face of the Mount Lofty Ranges, where the average annual rainfall drops from over 400 mm. to less than 300 within a few kilometres. In areas where environmental gradients are shallower, these two forms may be very closely associated, and very localized details of soil depth and texture become important in influencing which form occupies a particular site (Greenslade, 1976b).

I have made detailed surveys of several areas where two or more colour forms occur together. Maps of these areas are provided in Chapter 3, under the names Dublin, Eudunda, Eyre Peninsula and Morgan (Figures 3.5, 3.6, 3.7, 3.9). The sympatry suggested by the overall distributions of forms is evident in these areas on a very small scale, and nests of two different forms can often be found within 100 metres of each other. The Eudunda populations include part of the sharp P/B boundary referred to earlier.

In order to examine the interactions between colour forms on the finest possible scale, an area near Morgan (about 150 km. northeast of Adelaide) was chosen in which populations of P, B, and V occur within an area of approximately 600 x 1000 metres. This area is enclosed in a small rectangle in Figure 3.9, and shown in detail in Figure 2.5*. A striking feature of this map is that the three forms occur in distinct areas - they are not unsystematically intermingled. P dominates the

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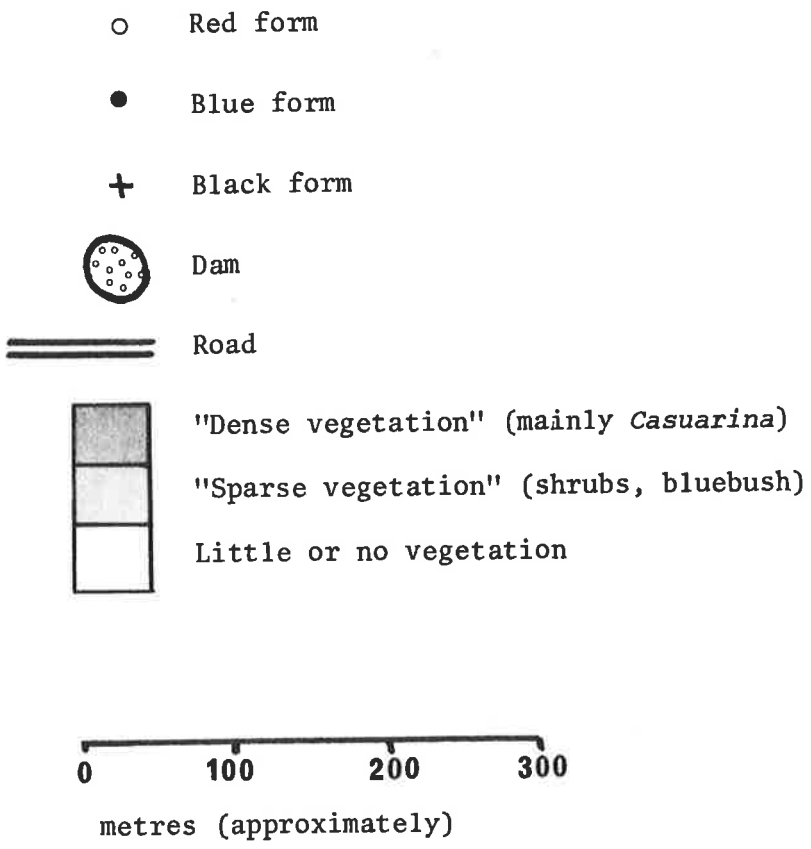
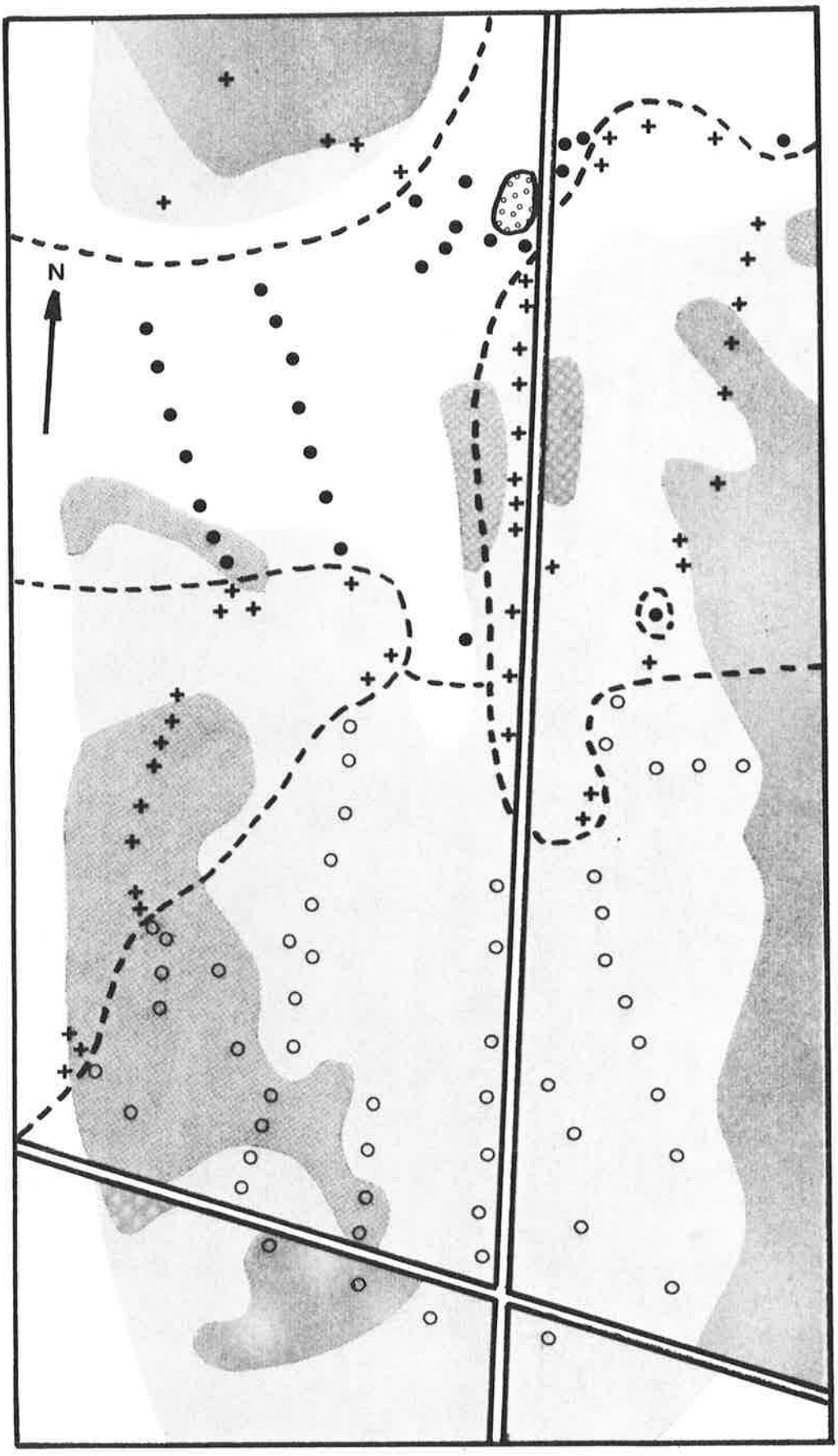
*This field work was done jointly with Dr. P. J. M. Greenslade.

Figure 2.5.

Location of meat ants in an area near Morgan, South Australia. Each point represents the location of a worker or a nest of the form indicated. Dashed lines enclose areas occupied by each form.




N

southern half of the area, and reaches extremely high densities.

V occupies three separate parts of the areas shown, to the east, west and north. The western V population is shown in its entirety, since the western boundary of Figure 2.5 marks the edge of a large area of pasture, in which meat ants forage but do not nest. Separating these three areas from each other are areas occupied only by B. A single record of B in a V population to the east is the only exception to the integrity of the areas occupied by the three colour forms.

It is also apparent from Figure 2.5 that the three forms occupy adjacent, but not overlapping territories. Territorial boundaries between adjacent colonies of the red form have been known for some time to be marked by a characteristic "confrontation" behaviour. The workers from opposing colonies examine each other carefully with their antennae, and exhibit a hostility display which has been described as a "stiff-legged walk". The body is raised high off the ground by straightening the legs, and the gaster is elevated higher still. The body quivers and movements are jerky and sudden, although contact rarely involves any physical injury (Duncan-Weatherley, 1953). I have also observed very similar behaviour between adjacent colonies of B. This behaviour usually makes it clear where territorial boundaries are, and it has been used in drawing these boundaries on several maps (*e.g.*, Figure 2.6). In cases where confrontation is not occurring naturally, it can be induced, by placing an ant from one colony in the territory occupied by another. Some or all of the features of normal confrontation are then shown when ants from the two colonies meet, although the hostility in this artificial situation may be more intense than normal.

When adjacent territories are occupied by ants of two different colour forms, the result is quite different. The ants physically attack each other, biting and pulling at appendages, and the ground is often littered with dead and dismembered ants of both forms. This behaviour

occurred where P and V come into contact, in the southwestern part of the area shown in Figure 2.5. Hostility of this kind has also been observed elsewhere, between P and B in several areas near Adelaide, and between P and Y in captivity (Greenslade, 1974c, and personal observations). The P and B ants in Figure 2.1 are manifesting this hostility, by grasping each other by the mandibles. Forde (1972) also noticed that inter-form aggression was more violent and hostile than intra-form, in the Flinders Ranges, but in captivity all interactions were of the violent type. Anonymous (1956) reports that competition between colonies is usually symbolic, but that in the case of *sanguineus*, "the nest site is often red with the bodies of ants killed in combat", so this form may be exceptional. Tepper (1882) describes many ants (obviously referring to meat ants) being killed when two "hostile districts" meet, but it is not clear whether they are of the same or different colour forms.

The violent hostility between colour forms partially accounts for their micro-geographic separation. The result of this type of interaction could be the destruction of one colony by theother, or as is more commonly observed, a slight contraction of territory by both, so that an uninhabited zone is left between them, and interactions are avoided.

Figure 2.5 also shows ecological differentiation between the forms. B occupies almost exclusively the areas shown as carrying little or no vegetation. These are overgrazed patches of low (30 cm., or less) shrubs, with large expanses of bare or lightly grassed soil, and very few trees. The areas where there is a reasonably dense growth of trees, or shrubs with scattered trees, are occupied by V and P. The classification of vegetation zones used is a subjective one, based on ground observations and examination of an aerial photograph. Nevertheless, the ability of B to survive in areas where P and V cannot is shown on this small scale, as it is on a broad geographic basis.

2.5. Mating and colony founding

Greaves and Hughes (1974) and Hughes (1974) have provided a detailed description of the founding and development of meat ant nests, based on observation of the red form near Canberra. Winged males and females are produced in late summer, and mating usually takes place in spring (October). The environmental conditions necessary for the initiation of a nuptial flight appear to be quite well defined. The ground must be wet from recent rain (in the previous 72 hours), the temperature must be close to 21°C, and the sun must be shining. Flights usually take place between two and three p.m., on calm days, and if these conditions persist flights may occur on several successive days, involving many nests.

Before and during the nuptial flight, the workers become very excited, and they apparently encourage the males and females to emerge from the nest and take flight. Mating takes place high in the air, while large numbers of males and females are on the wing simultaneously. Mated females return to the ground, shed their wings, and search for a nest site. Each then attempts to make a small nest, consisting of a shaft 5 to 10 cm. deep, with a chamber at the bottom.

The queens lay their first batch of eggs about a month after mating, and the first workers appear after approximately three more months. It may then take four more years for the colony to reach a total of 2000 workers, and to produce a new generation of males and females.

During this whole process, males and females are subject to heavy predation from birds and lizards, and females are also attacked by meat ants from existing colonies, both before and after the establishment of a new nest (Wilson, 1971; W, 1974; Greaves and Hughes, 1974). Heavy mortality continues under the combined effects of predation, disease and starvation, and the successful establishment of a new colony in an

area already occupied by meat ants is probably unusual.

However, when a new area becomes available to them, meat ants colonize it quickly. When the city of Canberra was established during the 1920's many suitable nest sites were created in an area where meat ants had previously not existed (Greaves, 1973). These were quickly occupied and incipient colonies continue to appear in areas where the existing nests have been destroyed (Greaves and Hughes, 1974). Colonizing ability is also indicated by the fact that meat ants often occupy every available nest site in an area. These sites often include man-made features such as roadsides, cleared areas of forest, and footpaths (Greaves, 1971, 1973).

The establishment of these new nests in disturbed areas is achieved by newly mated females. Meat ant queens are approximately 1.5 cm. long, and have large wings. These features suggest they are strong fliers, and that they may be able to travel considerable distances in search of a nest site, although a thorough investigation of queen behaviour has not yet been made.

2.6. Nest structure

The labels of preserved meat ants in museums rarely carry any information about the type of nest from which the specimens were collected. However, some aspects of general biology and especially nest form, can be particularly valuable in distinguishing species of social Hymenoptera (Richards, 1951).

Some meat ants nests are very conspicuous, and have attracted comment from many authors. These nests have earned meat ants the alternative common names "mound ants" (*e.g.*, Clark, 1924) and "gravel ants" (Brewster *et al.*, 1946). The nests consist of several to many entrance holes in a well defined group, usually in a mound composed of small stones, soil, or fragments of plant material. Greaves (1939) describes one such mound (of P), over ten metres in diameter and half a metre high, containing over 1000 holes, and Beattie (1972) mentions a mound which was "estimated to contain some 40 tons of material". A more typical mound would be one to two metres in diameter, with about 30-40 holes. In some cases, the mound is not well-developed, and the nest is marked only by a circular or oval shaped area of bare ground, but even then there is usually some decoration of the nest surface with fine gravel or other material. Some of this material is visible in Figure 2.2. Its function is not known, but it may serve as a dew trap, or an aid in regulation of nest temperature (Ettershank, 1971).

These mound nests may be very long lived, apparently surviving prolonged periods of erosion of the surrounding soil (Greenslade, 1974a). The considerable age of some of these mounds has also been noted by Beattie (1972) and Greaves (personal communication), while W (1974) reports one colony occupying the same site for more than 70 years. Ettershank (1971) found that these mounds allowed the maintenance of high relative humidity within the nest, and aided in the regulation of its temperature.

When an individual mound nest is excavated, little of its structure can be discerned (Duncan-Weatherley, 1953). However, a more efficient way of examining the internal structure of a meat ant nest is to make a cast of it, either from lead (Ettershank, 1968) or paraffin wax (Salinitri , 1976). This technique has revealed that separate entrance holes on the surface are usually not connected underground (Ettershank, 1968). Marked ants which are artificially confined to the area around one hole do not emerge from other holes, even after prolonged starvation (Hilditch, 1968). Each new hole is therefore started from the surface and leads into a separate system of galleries. Furthermore, although the workers from these separate gallery systems mingle freely during the day, each appears to return to the same hole each night (Hilditch, 1968). The nest is not a unified whole, but is structured both physically and behaviourally.

Compound nests of this type, with varying degrees of mound development and gravel or other decoration, are built by the forms P, V, Y, and DY.

The nests built by B are quite different. They consist of single isolated entrance holes, sometimes as small as 2 mm., in diameter, and sometimes with a 1-2 cm., high turret. Several such nests may jointly form a colony and exchange workers, but the individual holes are never closer than about half a metre (Greenslade, 1970). Some nests of O are of this simple type while others consist of a loose aggregation of holes closer together than this. They are sometimes surrounded by gravel but there is no distinct mound (Greenslade, personal communication).

The nests built by SP are also variable. In South Australia they consist of simple single holes (sometimes with turrets) as with B, while in southwestern Western Australia, where SP is the only form present, compound nests are built. These two nest styles seem to intergrade, with

some loose aggregations of holes and some large fairly well defined mounds (Clark, 1924; Majer, personal communication).

Sanguineus also shows variation in nest architecture, from single holes to large mounds (Greaves, 1971) and is often found nesting in termite mounds.

2.7. Colony and population structure

Mature colonies of meat ants often contain several to many separate nests, which exchange workers across the soil surface along what Duncan-Weatherley (1953) referred to as "common trails". These are often visible as permanent pathways, cleared of vegetation for a width of several centimetres, and carry heavy traffic of ants in both directions.

It is sometimes clear which is the main nest in such a colony and which are "satellites", but more often a colony consists of a series of nests of varying sizes, and it is not possible to decide which of them is the oldest. The existence of multi-nest colonies and trails between nests has been described by Tepper (1882), Froggat (1907), Greaves (1939), Brewster *et al.*, (1946) and many subsequent authors.

It was shown previously that a mound nest is not a single unstructured unit, but that each hole leads to a separate system of galleries within the general body of the nest. The opening of a new hole at some distance from the nest therefore involves only a quantitative change in behaviour, and the establishment of a satellite nest in this way can often be observed in progress. New satellite nests are established near food sources such as trees, as the population of a colony increases and its territory expands.

Other aspects of colony organization have been revealed by detailed study of a population near Adelaide (Greenslade, 1975a, 1975b). It is apparent from this work that a colony may gain nests by taking over those previously occupied by another colony. Nests are often abandoned due to lack of food supply, human interference, or encroaching shade. The surrounding colonies are then able to expand into the abandoned territory, and one of them may take possession of the empty nest. When a colony abandons a nest, it need not become extinct. A multi-nest colony

may contract its territory by abandoning one nest while continuing to occupy others. A further possibility is that a single-nest colony may establish a satellite nest and move into it, abandoning the previous nest.

When all these factors are combined, a population of meat ants is seen to include a mixture of new, growing, mature, declining, and abandoned nests. The details of the arrangement of nests and colonies may change with the seasons, or with short or long term changes in the environment, but in the absence of human interference the broad pattern of nests may be stable for quite long periods (Greenslade, 1975a, 1975b).

Most of the information presented so far refers to P, although it is possible that Ettershank (1968, 1971) included nests of Y in his "South Ita Sandhills" population. Very little is known of the colony organization of forms other than P. Greenslade (1970, Figure 1) shows a map of nests and colonies which apparently includes both B (colonies A-F) and V (Colony G) (Greenslade 1974b) in an area of northern South Australia. Both of these forms occupied multi-nest colonies, but in the case of B, each nest was a single hole. The nests of V consisted of 8-11 holes, in bare areas of ground decorated with pebbles and soil crumbs. The existence of multi-nest colonies of these forms in this area is completely consistent with subsequent observations in many other areas.

In order to examine B and V colonies more fully, a small study area near Morgan was chosen, in which they both occur. Figure 2.6 is a map of the nests and colonies found.* The area of Figure 2.6 is within that shown in Figure 2.5, and its location can be reocognized by the dam, which is shown in both. The B nests in this area have small populations

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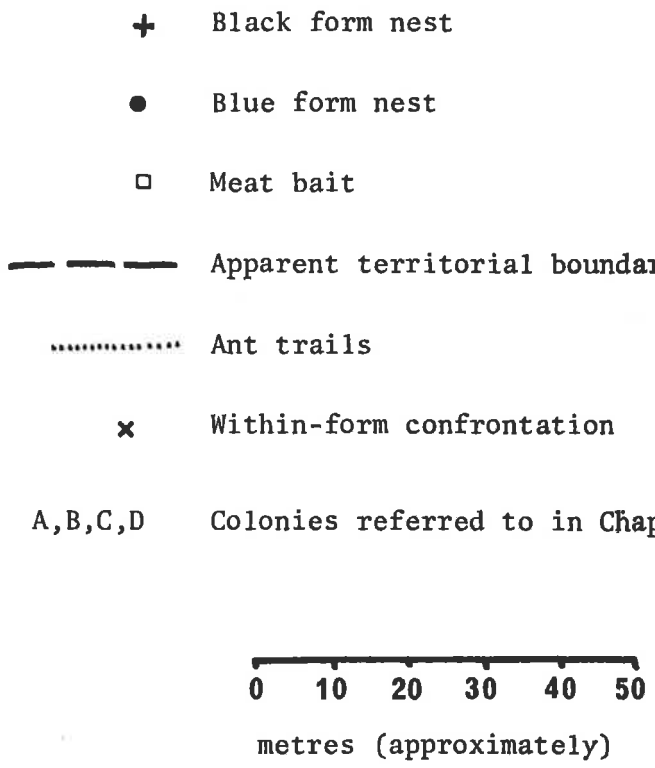
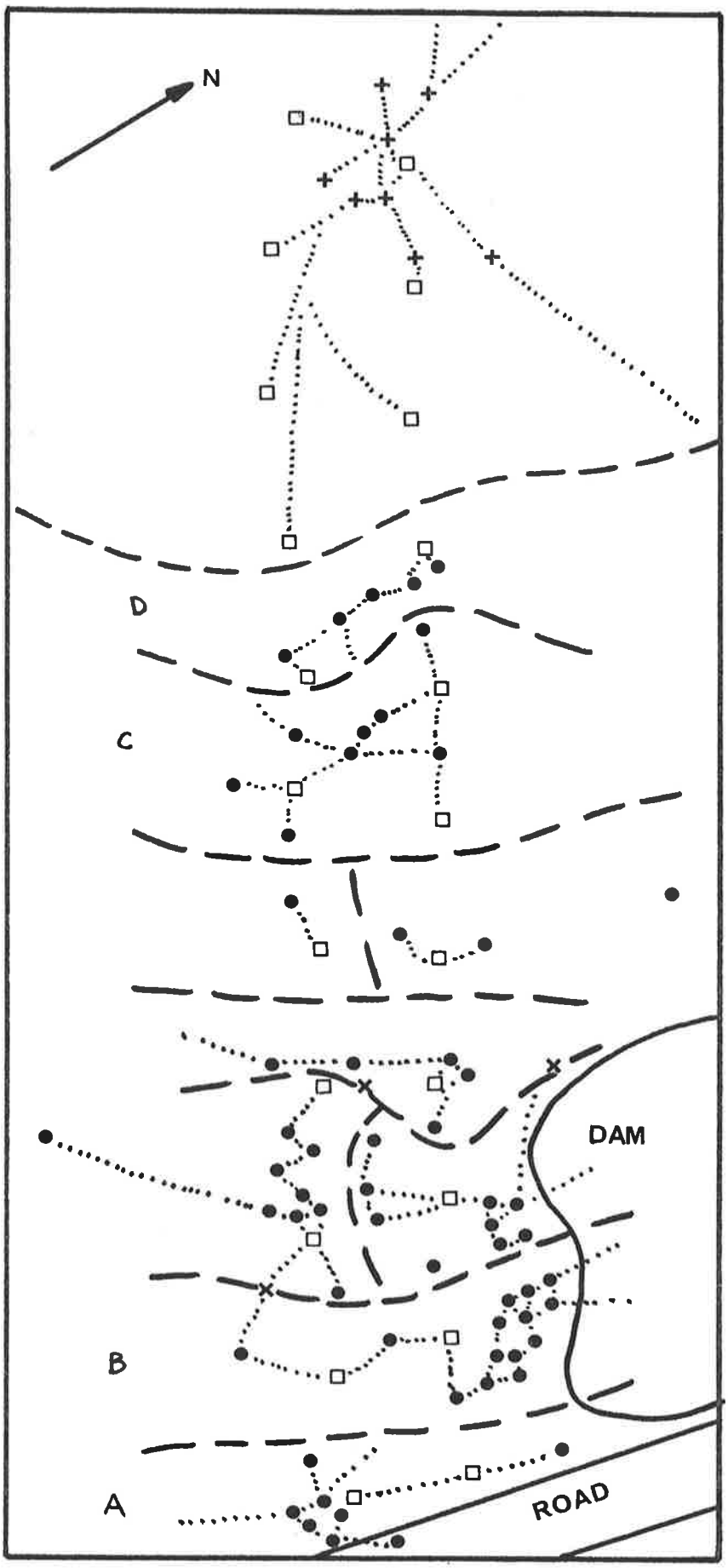
* This field work was carried out jointly with Dr. P. J. M. Greenslade

Figure 2.6.

Map of nests and colonies of the blue and black forms of meat ants at a site near Morgan, South Australia.




N
D
C
B
A
DAM
ROAD

which are not very active, so it was necessary to place meat baits on the ground, and to follow ant trails from them back to the nests.

Many trails of B were found, either leading from baits or trees back to the nests, or connecting nests. In several places, territorial confrontations similar to thoseof P, were observed. These, and the existence of patches of ground with no visible meat ant traffic, led to the construction of the territorial boundaries shown in the Figure.

The B nests shown occupy an area referred to earlier as carrying "little or no vegetation". It consists of large areas of bare soil, subject to surface erosion, with scattered clumps of grass and shrubs. There were several small trees beside the dam shown in the Figure, and the B colonies which foraged on them showed a rather higher concentration of entrances than those in less productive areas.

At the top of Figure 2.6 there is a patch of small trees (mainly *Casuarina*), occupied by a multi-nest colony of V. This colony contained eight nests, ranging from 1 to 19 holes each. Foraging trails were formed from these nests out into the open areas for over 50 metres, to make use of the nearest baits. Although this took the V workers to within a few metres of B, they did not meet, and no direct interaction between these forms could be observed in this area.

Anonymous (1973) refers to colonies consisting of hundreds of nests, joined by permanent trails, in Western Australia. The maps in Figure 2.4 reveal that this could refer to either SP or V, or both, so multi-nest colonies appear to be developed by meat ants over wide areas.

The use of multi-nest colonies allows meat ants to respond to local variations in food supply and changes in vegetation type within

their territories, by the creation of new nests or the abandonment of old ones. It therefore allows them considerable flexibility in the exploitation of resources. The nature of these resources, the techniques which meat ants use to exploit them, and the interaction of meat ants with humans, are examined in the next section.

2.8. General biology and pest status

The principal food source utilized by meat ants is honeydew, excreted by species of Homoptera. The Homoptera involved include *Cometopsylla, Glycaspida, Eurymeloides* (Culley, 1969), *Eurymela fenestrata* (Viehmeyer, 1925), *Gascardia (Ceroplastes) destructor* (Majer and Livingstone, 1976) and *Australopsylla* (Greenslade 1970), as well as many species which have been mentioned but not specifically identified.

Meat ants also obtain nutrition directly from plants in the form of nectar and other exudates, as well as by consuming flowers and fruit from a wide variety of plants (many observations, including Clark, 1924; McKeown, 1945; Brewster *et al.*, 1946; Anonymous, 1956 Ettershank, 1971; Hughes, 1974).

Meat ants get their common name from their predatory and scavenging habits. They prey on virtually any insects they can catch, and specific examples from the literature include termites (Greaves, 1939; Greenslade, 1970), *Cactoblastis* larvae (Dodd, 1940), the psyllids *Cardiaspina* (Clark, 1964) and *Glycaspis* (Hughes, 1974), the eggs and nymphs of stick insects and cicadas (Hughes, 1974), and flies (Greenslade, 1970). Their appetite for meat is not confined to insects, and carrion of all kinds is included in their diet, including dead insects and other invertebrates, as well as dead vertebrates of all kinds, and food scraps and litter left by humans. Their omnivorous nature, large size, and high population densities, make meat ants dominant members of ant communities, and they have a marked influence on the abundance of other species of ants (Greenslade 1973, 1975c, 1976a; Matthews, 1976).

Permanent trails lead from the nests to one or more trees in the territory occupied by each colony. These trails are used by "tree

foragers", which attend the honeydew producing Homoptera and obtain other exudates from the trees. Each tree forager visits only one tree (Hilditch, 1968), and may even have its activities confined to one part of one tree (Culley, 1969). Tree foragers do not forage on the ground (Doube, 1967). The area covered by ground foragers is also subdivided into individual areas, and the entire ground surface is covered thoroughly several times each day. The discovery of a large item of food on the ground (*e.g.*, carrion) results in the recruitment of extra ground foragers along a temporary trail, which disappears when the food is exhausted.

Thus, despite the apparent inefficiency and clumsiness of meat ants noticed by Duncan-Weatherley (1953), they have achieved a high degree of social organization which allows them to successfully exploit all food sources available to them.

Foraging activity is influenced by several environmental variables. There is little or no activity at night, although ants can be found on the nest surface and trees, as well as carrion sources (Culley, 1969; and personal observations). The onset of activity in the morning is initiated by the change from darkness to light (Majer and Livingstone, 1974) and after that, the amount of activity is related to temperature (Mitchell, 1966; Greaves and Hughes, 1974; Majer and Livingstone, 1974) and humidity (Nel, 1967). Workers lose water quickly and they are not active during the hottest part of the day, or above about 35^{o}C. Sensitivity to dehydration is probably alleviated to some extent by the use of honeydew as a water supply (Nel, 1965), and it is known that tree foragers descending a tree after feeding on honeydew have significantly larger abdomens (Mitchell, 1966), and are more resistant to dessication (Doube, 1967), than those ascending.

Several factors combine to make meat ants occasional pests to humans. Their omnivorous nature, colonizing ability, large numbers, and preference for cleared sites for the building of nests, all equip them to exploit human habitations. Their status as a domestic pest near buildings is described by Greaves (1973) and Anonymous (1973). They may also become an agricultural nuisance in several ways.

Meat ants' habit of attending Homoptera in trees means that they may encourage the build up of scales, lerps, *etc.*, in orchards, and may reduce the efficiency of predators which would otherwise destroy them (Greaves, 1939; Majer and Livingstone, 1976). Also, in the search for nectar and other plant foods, they may destroy blossoms and reduce the yield of fruit trees (Clark, 1924; Greaves, 1939; Anonymous, 1956).

Meat ants have also been observed as predators of insects introduced for the biological control of weeds - *Chrysolina varians* on St. John's Wort (Currie and Fyfe, 1938), and *Cactoblastis cactorum* on prickly pear (Dodd, 1940).

Attempts have been made to control meat ants by the use of various poisons, with varying degrees of success. Greaves (1939) used poisons ranging from calcium cyanide to crankcase oil, and other substances used include carbon bisulphide, kerosene, calcium cyanide, DDT and other chlorinated hydrocarbons, and organic phosphates (Clark, 1924; Summerville, 1929; Greaves, 1939; Anonymous, 1956; Greaves, 1973).

Whatever the substance used, the attack is directed at the nest. However, several aspects of meat ant behaviour make their control difficult (Greaves, 1973). Entrance holes are not connected underground, so poison must be applied down every hole in a large nest. In a multi-nest colony, every nest must be found and treated, or treated nests will

quickly be re-occupied from other nests in the colony. Furthermore, even if a whole colony is effectively treated, the area may be taken over by ants from surrounding colonies. Meat ants may also establish new nests in a treated area through the action of newly mated queens. It is therefore necessary to re-survey and re-treat an area every two or three years to achieve effective control (Greaves, 1973).

Meat ants are attacked by a number of predators including the larvae of beetles *(Spallomorpha)* (Moore, 1974) and moths *(Cyclotorna)* (Matthews, 1976) which can be found in their nests (Greaves and Hughes, 1974). They are also attacked by other ants and a number of species of spiders, some of which appear to be meat ant mimics (Greenslade, personal communication). None of these predators shows any sign of having a significant effect on meat ant numbers, or of making any contribution to their control.

Lofgren *et al.*, (1975) point out the possibility of using pheromones to control fire ants *(Solenopsis)*, possibly through the construction of false pheromone trails to poison baits. Work on the chemistry of meat ants has shown that they produce a number of unusual substances, so this possibility may exist here also (Cavill and Ford, 1953; Cavill *et al.*, 1956; Cavill and Locksley, 1957; Cavill and Ford, 1960; Cavill and Hinterberger, 1960).

2.9. Overview

The general picture of meat ants which emerges from the study of a large amount of literature, is that they are a numerous, dominant, and highly successful group. Flexibility in colony structure, efficient social organization, and ecological generalization, allow them to exploit a wide variety of resources. A combination of territorial hostility and cononizing ability means that meat ants are able to saturate suitable habitats, as well as quickly occupying new ones which become available to them.

The existence of colour differences between forms is now clearly established, and at least in some cases, these differences are correlated with variation in habitat and climatic factors, size, nest form, and behaviour. When taken together, these factors indicate that serious consideration should be given to the suggestion that the colour forms are separate species. Subsequent chapters describe evidence of several other kinds which has been collected, and which is generally consistent with this suggestion.

CHAPTER 3

MATERIALS AND METHODS

3.1. Populations sampled

Figure 3.1 is a map of South Australia showing the locations of places referred to in later sections. Ants were collected from a number of different areas which are described below in alphabetical order. In the descriptions and maps to follow, the only nests included are those from which ants were collected for electrophoresis. In most of these areas many other nests are known, and have been used in compiling overall distribution maps such as those in Chapter 2.

3.1.1. Adelaide Hills

Figure 3.2 shows the locations of red form nests sampled in Adelaide and the nearby Adelaide Hills. The area shown also includes an isolated population of the blue form at Hallett Cove. This population is more than 60 km. from the nearest other occurrences of this form, near Mannum and Dublin, and may be a relic of a once wider distribution (Greenslade, 1974c). It is surrounded by red form nests, four of which are included in the Adelaide Hills sample.

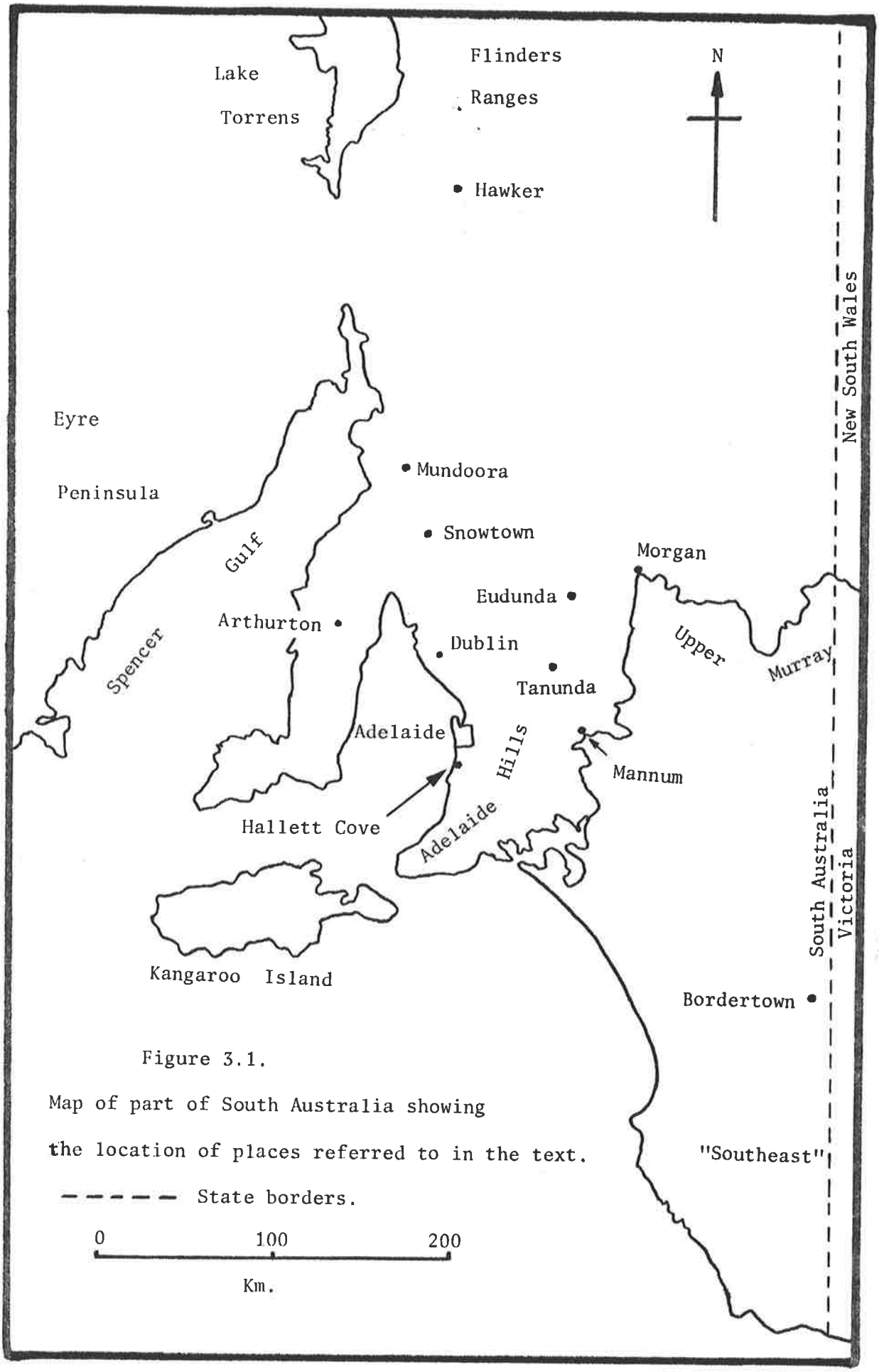


Figure 3.1.

Map of part of South Australia showing the location of places referred to in the text.

– – – – – State borders.

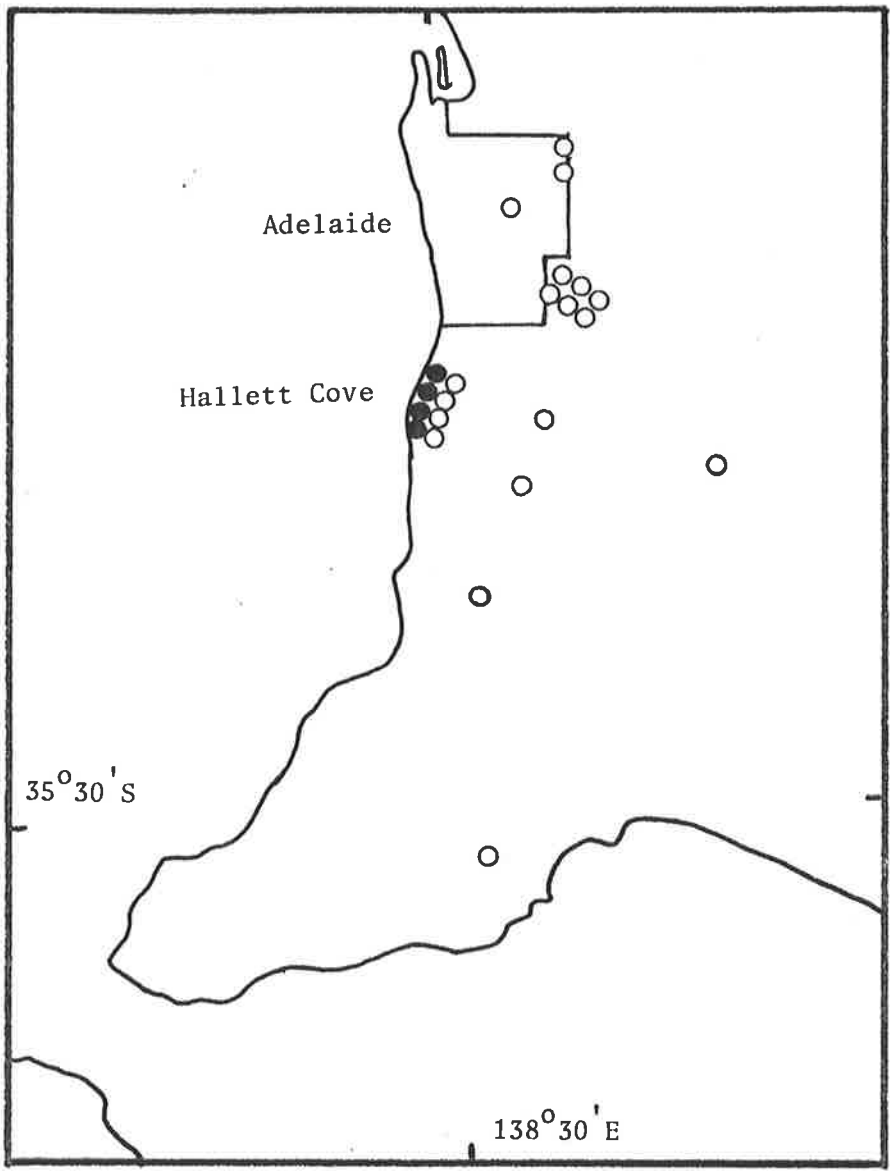


Figure 3.2.

Collection localities for the "Adelaide" and "Hallett Cove" populations.

○ Red form nest

● Blue form nest

3.1.2. Arthurton

Figure 3.3 shows the nests from which ants were collected near Arthurton, on northern Yorke Peninsula. The red form reaches very high densities along roadsides in this area, and is the only form to occur in the area shown. Populations of the blue form are located approximately 20 km. from Arthurton, both to the northwest and east (Greenslade, 1976b).

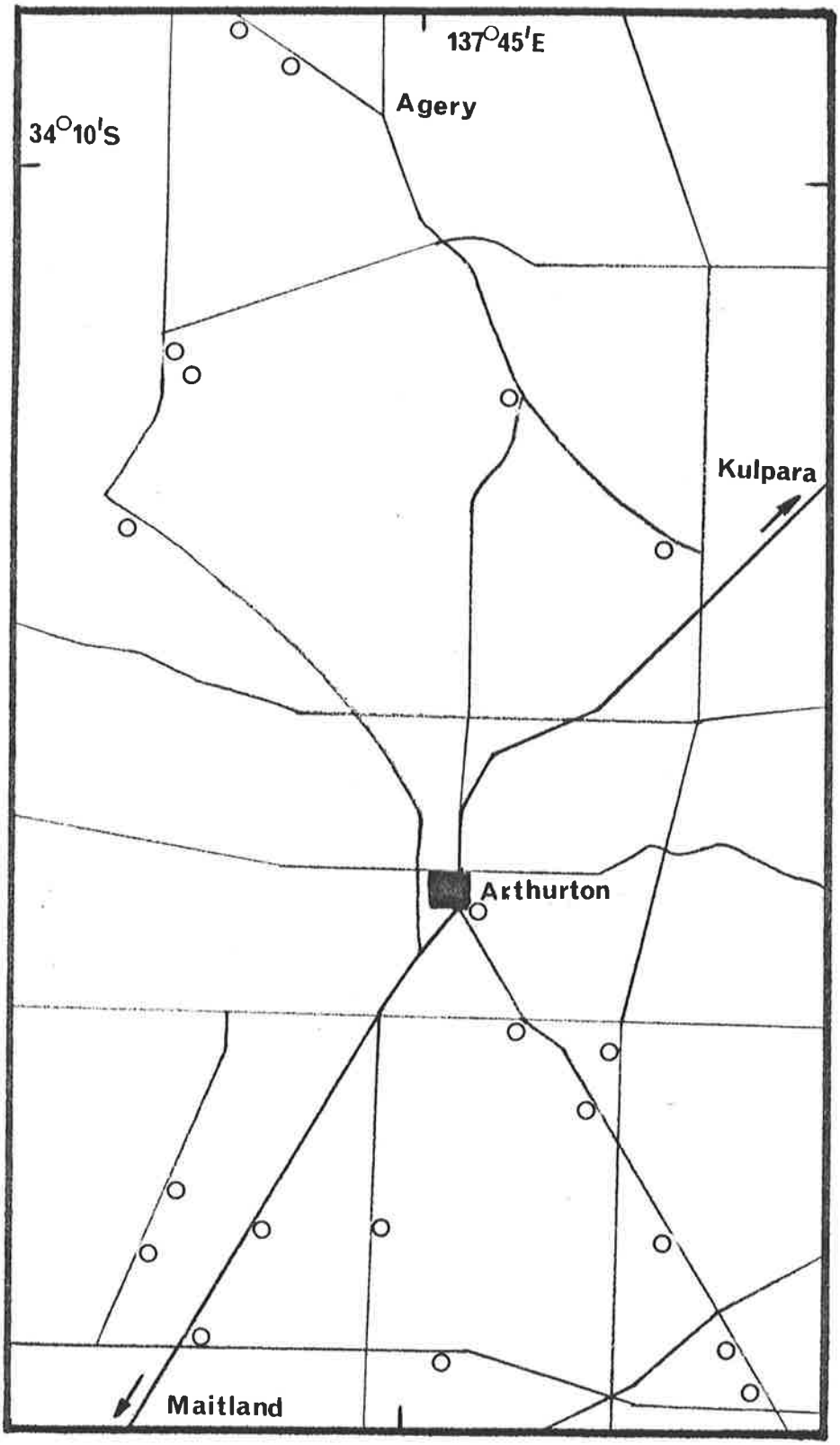


Figure 3.3.

Collection localities for the "Arthurton" population.

O Red form nest.

——— Roads.

0 1 2 3 4 5

Km.

3.1.3 Barossa Valley

A population of the red form collected in and near the Barossa Valley, centred on the town of Tanunda, is shown in Figure 3.4. This area is located between the "Adelaide" and "Eudunda" populations, and Figure 3.4 includes a small part of the latter to the northeast. Once again, the red form is the only one to occur in the area shown.

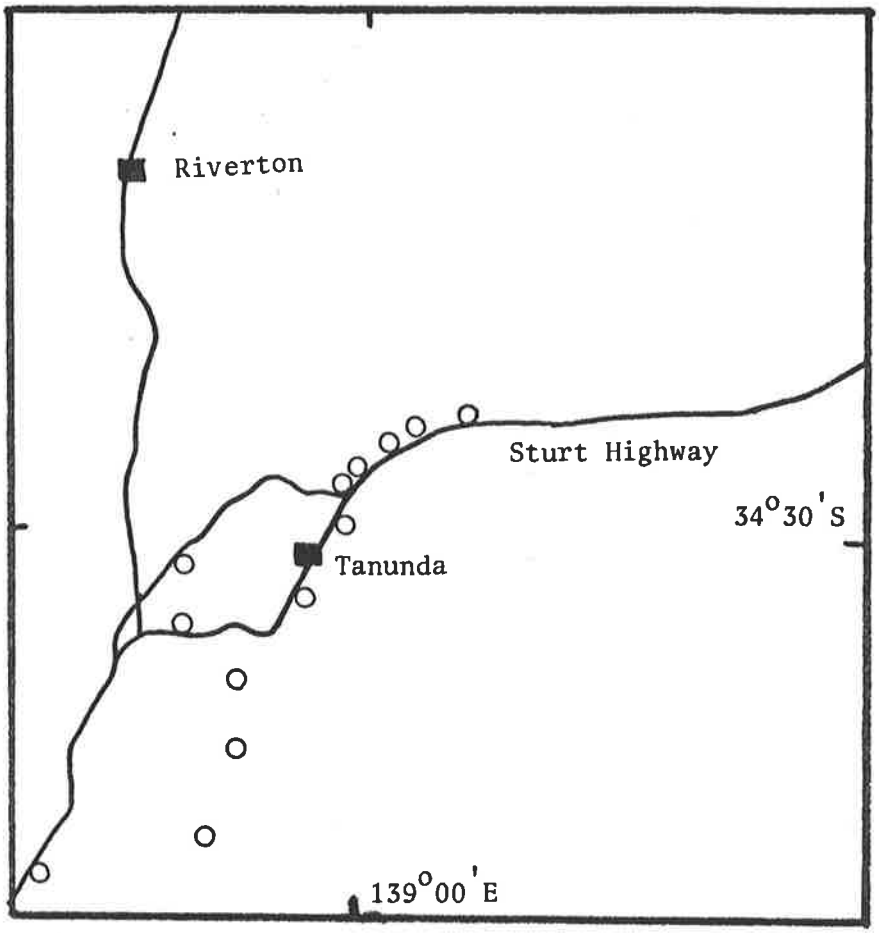


Figure 3.4.

Collection localities for the "Barossa Valley" population.

O Red form nest.

—— Roads.

0 10 20 30 40 50 60

Km.

3.1.4. Dublin

Figure 3.5 shows the Dublin populations. This is an area of close sympatry between the red and blue forms, and both reach moderate densities along roadsides, and around the few remaining patches of native vegetation. A total of 17 nests of each were collected within an area of about 13 x 15 km. In some cases this involved collections of the two forms from nests separated by no more than 100 metres.

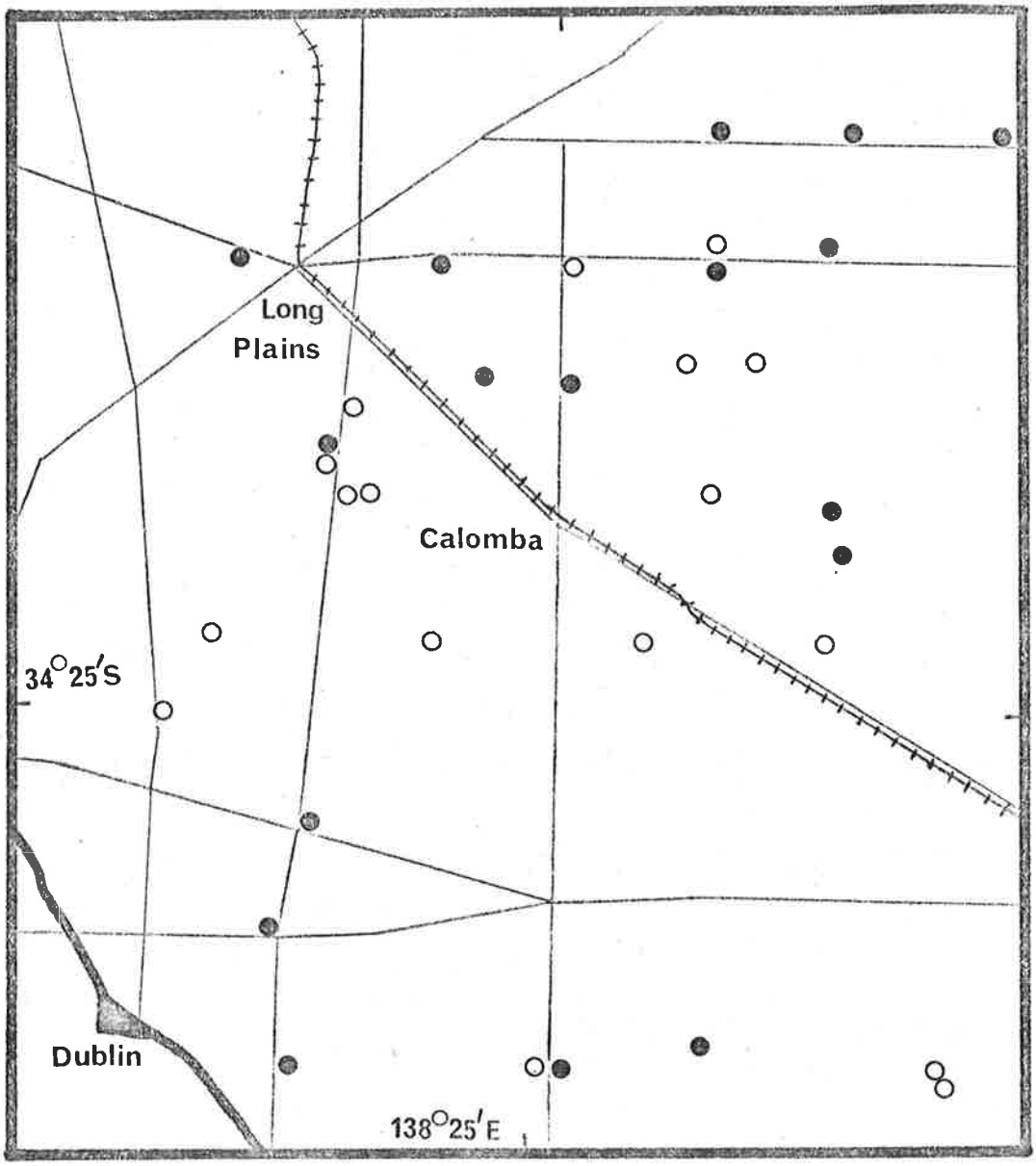


Figure 3.5.

Collection localities for the "Dublin" populations.

○ Red form nest.

● Blue form nest.

Railway.

Highway.

Other roads.

3.1.5. Eudunda

Greenslade (1974c) describes a sharp boundary between the red and blue forms which runs along the eastern margin of the Adelaide Hills. The collections from near Eudunda shown in Figure 3.6 were designed to straddle this boundary. The boundary runs approximately from upper left to lower right on the map, with the red form predominant in the south and west, and the blue form mainly confined to the north and east of the area shown. The boundary is actually a zone of uncertainty within which the occurrence of single nests of each form is determined by highly localized features of soil and vegetation. Once again, there is very close sympatry in this zone, and red-blue conflicts have been observed in the northern part of this area (Greenslade, 1974c; and personal observations).

Figure 3.6.

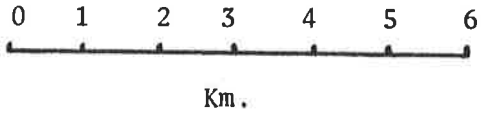
Collection localities for the "Eudunda" populations.

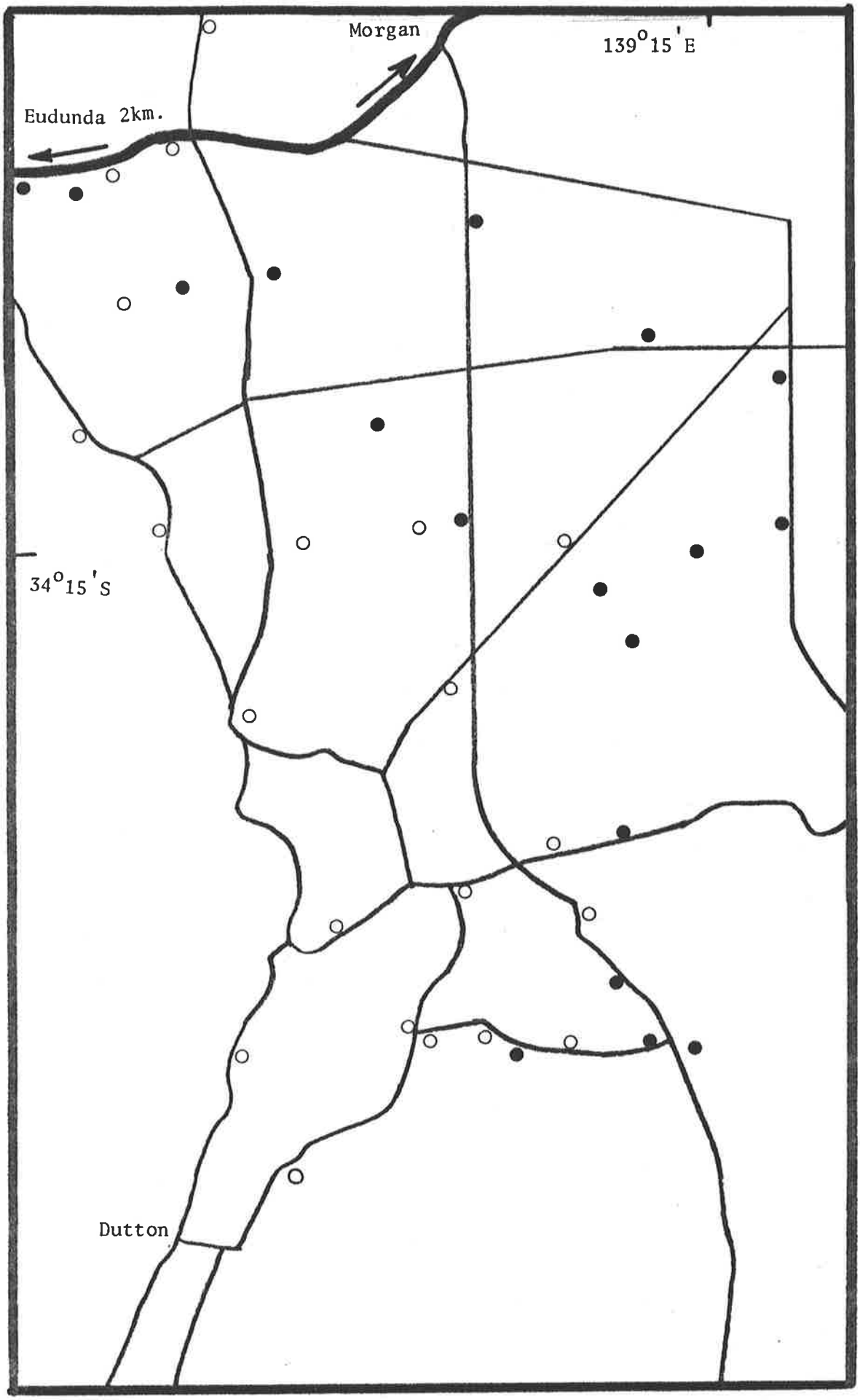
○ Red form nest.

● Blue form nest.

━━━ Highway.

——— Other roads.



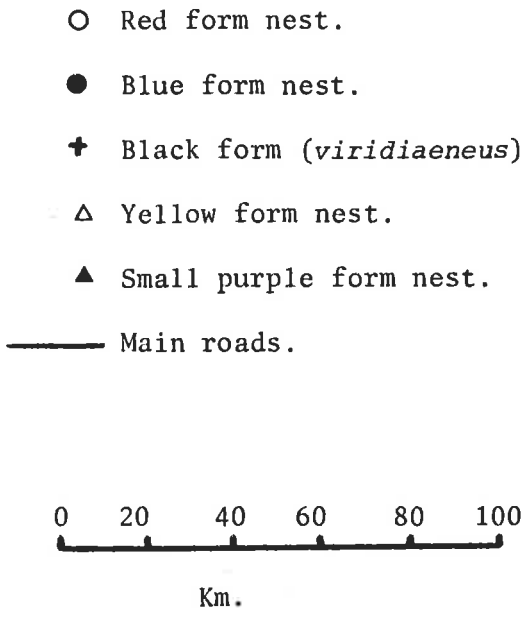

Morgan
139°15'E
Eudunda 2km.
34°15'S
Dutton

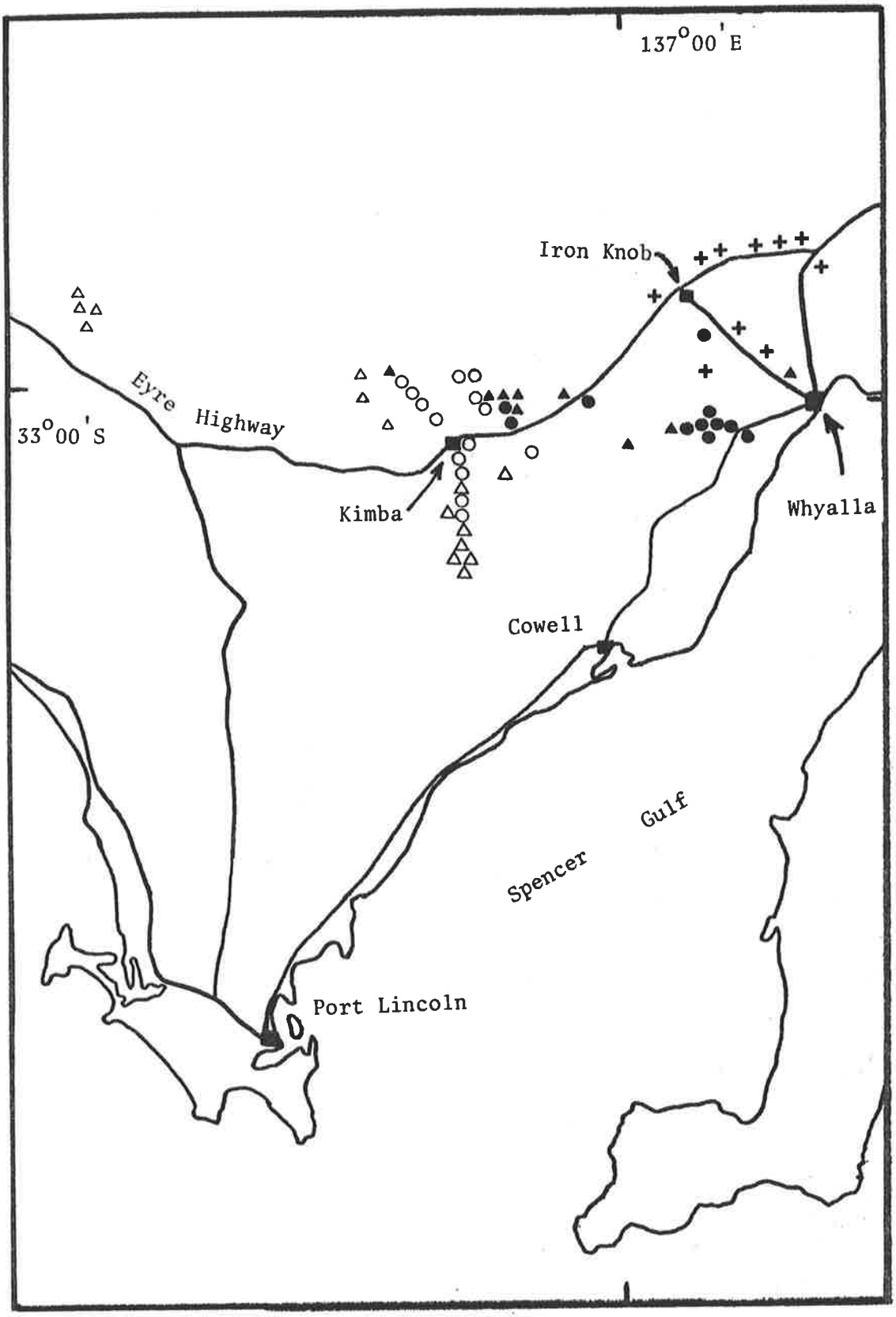
3.1.6. Eyre Peninsula

Figure 3.7 shows the location of nests sampled on Eyre Peninsula. Ants of five colour forms are included, compared with a maximum of three for any of the other areas visited. The inaccessible nature of much of the area shown, and the fact that meat ants are not common in the poor soils and sparse vegetation it contains, contribute to making the area covered substantially larger than for any of the other collections. In various parts of the area, combinations of two or three colour forms can be found in close proximity, and in general it is difficult to predict which form or forms will be found at a particular site.

Figure 3.7.

Collection localities for the "Eyre Peninsula" populations.



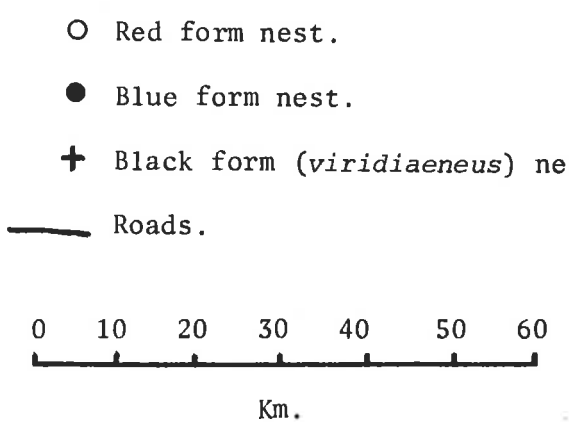

137°00'E
Iron Knob
Eyre Highway
33°00'S
Kimba
Whyalla
Cowell
Spencer Gulf
Port Lincoln

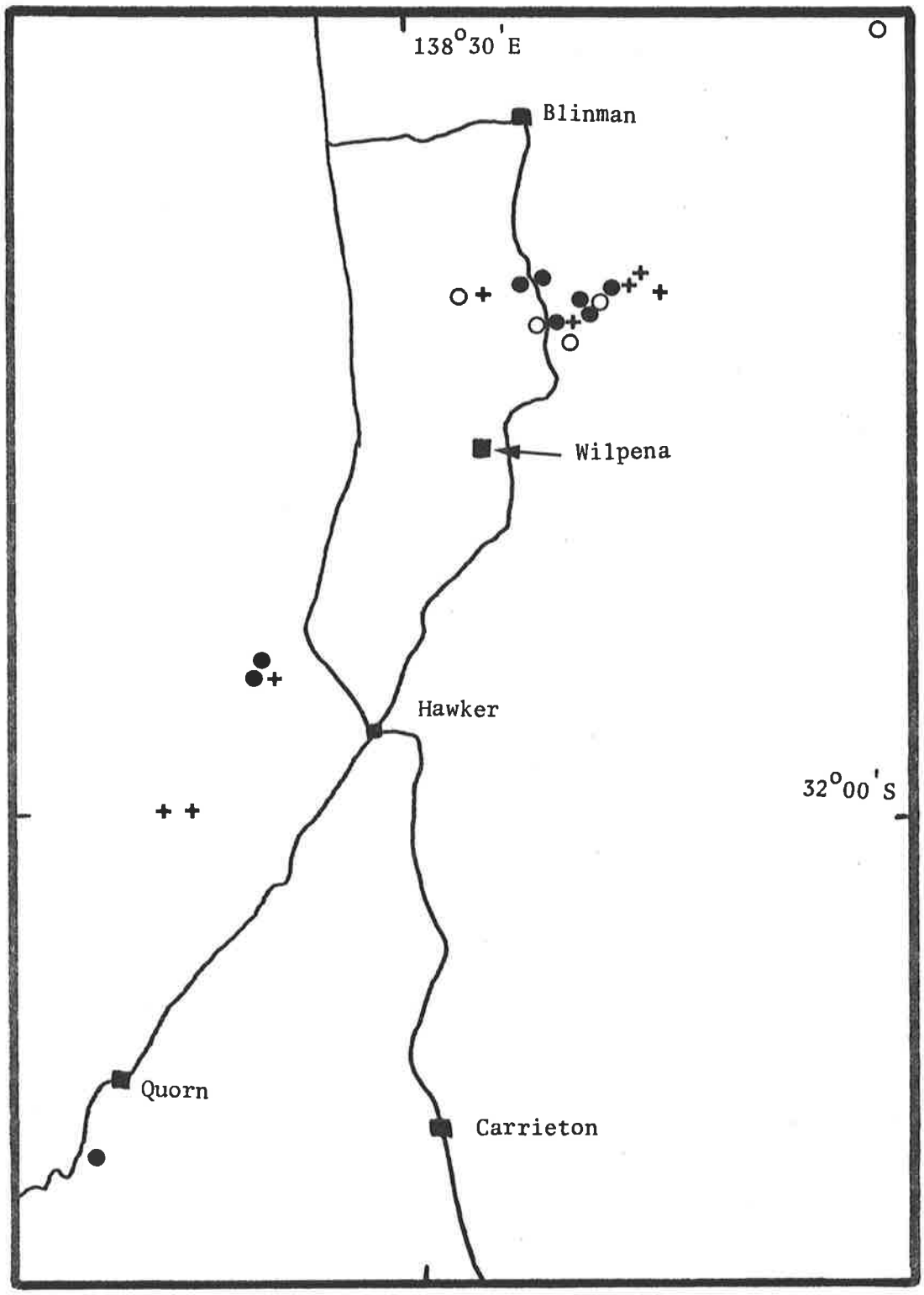
3.1.7. Flinders Ranges

The Flinders Ranges contain dense populations of the red, blue and black forms of meat ants. The localities from which collections were made are shown in Figure 3.8, and are mainly from the Flinders Ranges National Park. Once again close sympatry is evident, involving all three colour forms, and all of them can be found within 500 metres of each other in some places.

Figure 3.8.

Collection localities for the "Flinders Ranges" populations.




138°30'E
Blinman
Wilpena
Hawker
32°00'S
Quorn
Carrieton

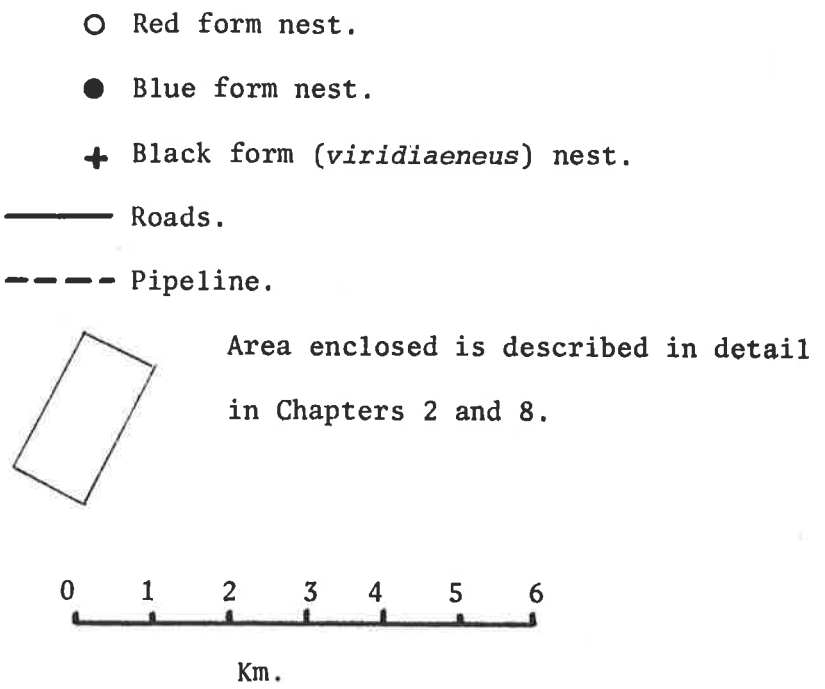
3.1.8. Morgan

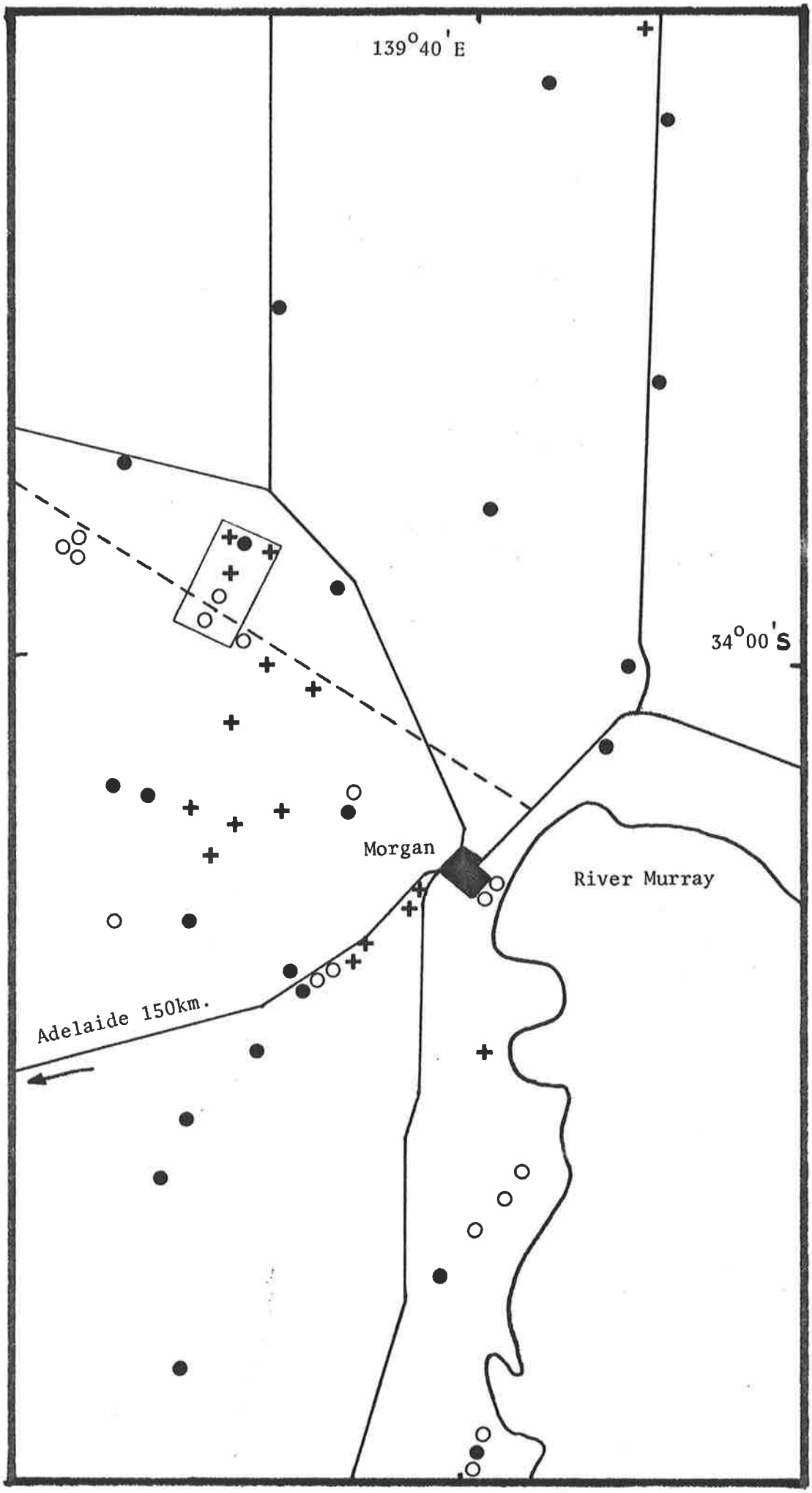
The red, blue and black forms also occur together in an area around Morgan, as shown in Figure 3.9. This area is surrounded by arid areas occupied mainly by the blue form. The red form is apparently able to occupy this type of country only where watercourses allow the growth of large trees, and supply moisture through the soil. In this case the River Murray supports populations of the red form which penentrate far into dry areas both upstream and downstream of Morgan.

The three forms occur in very close contact in parts of the area shown. One of these sites has been described in detail in Chapter 2, and is enclosed by a small rectangle on Figure 3.9.

Figure 3.9.

Collection localities for the "Morgan" populations.




139°40'E
34°00'S
Morgan
River Murray
Adelaide 150km.

3.1.9. Mundoora

The small purple form of meat ants occurs from Western Australia across the Nullabor Plain, and stops at Spencer Gulf (Figure 2.4). The single known exception is a small enclave east of the Gulf, at Clements Gap, near Mundoora (see Fig. 3.1). This population is within a few km. of both red and blue form populations. Collections were made from several single hole nests in this population, which seems to occupy an area of about a hectare.

3.1.10. Snowtown

Another small collection was made 15 km. east of Snowtown (see Fig. 3.1). The samples consist of four nests of the blue form and one of the red form, from a 600 metre stretch of road. The significance of this, population, and the one from Mundoora, is described in more detail in Chapter 4.

3.1.11. Southeast

A small collection was made from five widely scattered nests of the red form in the southeast of South Australia. This is the closest approach to an area occupied by only a single colour form, out of all the populations sampled. The nearest nests of another colour form are those of the yellow form, which occurs on sandy soils north of Bordertown (see Fig. 3.1).

3.1.12. Upper Murray

The smallest collection made consisted of four nests of the red form, from the Upper Murray region of South Australia, near the Victorian border. The red form exists here only very close to the river, in a region surrounded by populations of the blue form.

3.2. Collection Methods

Once a nest was located, a collection was made in various ways which depended on the local circumstances. In most cases ants were collected in a plastic screw top jar, of about 200 ml. capacity. The lid had a diameter of 8 cm. with a 2.5 cm. diameter hole in it. When this was inverted over an entrance hole of a nest a large number of ants immediately rushed into the jar and could be easily secured. In the case of multi-entrance nests, this procedure was repeated for several entrance holes and collections pooled. For less populous or active nests, especially those of the blue and small purple forms, less efficient procedures were used. Ants could be collected individually by aspiration or with forceps either from the nest or from the surrounding soil surface. In a few cases where the nest could not be found after prolonged searching, collections were made from a small area of ground, or from a single tree.

When the collection was complete, a ventilated lid was fitted to the jar, and it was placed in a dark humidified ice-box. Several hundred ants could be kept alive in each jar for several days under these conditions.

On return to the laboratory, dead ants were removed and the jars placed directly into a deep freeze at -30°C. Later, the ants were transferred to a smaller container for prolonged storage.

Some specimens from each nest were also preserved in 70% ethanol, and appropriately labelled with details of locality, nest form, date *etc.* Representative voucher specimens will be lodged with the Australian National Insect Collection, CSIRO, Division of Entomology, Canberra.

3.3. Sample preparation

When required ants were removed from the deep freeze and the gaster and legs removed and discarded. The head and thorax were placed in a labelled polypropylene centrifuge tube (0.6 ml. capacity, Kayline MCP-5505C) with two drops of distilled water. They were then individually ground with a Teflon plunger specially made to fit the interior of the tubes. This plunger was vigorously scrubbed with a wet cloth between specimens. Extracts were then returned to the deep freeze, and re-thawed when needed for electrophoresis. Immediately before loading, they were centrifuged in a Beckman 152 Microfuge for one minute. The supernatant was then loaded directly into slots in the gel.

The amount of water used in extraction was either reduced or increased when the ants were very small or very large, and was reduced to a minimum for males. Some specimens with very strong amylase activity could not be scored at the first running, so they were diluted with an extra drop of distilled water, re-suspended, and re-centrifuged before running for the second time.

Extraction in a dilute solution of Na_2EDTA, a Tris/citric acid buffer (pH = 7.0), or 0.87% Sodium chloride did not noticeably improve the resolution of gels. Specimens intended for Glutamate dehydrogenase typing were extracted in a 10^{-4}M solution of NAD (see Chapter 6). In this case NAD was also added to the gel and electrolyte buffers.

Prolonged storage at -30°C did not noticeably reduce the activity of amylase or esterase enzymes. For some other enzymes, especially Malic enzyme, activity decreased sharply on storage and repeated freezing and thawing.

3.4. Electrophoresis Methods

Electrophoresis was carried out horizontally in a refrigerator at 3°C. Gel trays were 155 x 200 x 6 mm. and each gel held 16 specimens. Starch used for some systems was either Electrostarch (used at a concentration of 11% starch) or Connaught (12%). Polyacrylamide gels (5%) were prepared using the TEMED-Ammonium Persulphate polymerization system. In both cases gels were prepared the day before use, and allowed to set overnight.

A number of different buffer systems were used for electrophoresis. In the following recipes, all quantities are per litre of single strength solution. For abbreviations, see Appendix 5.

I Tris/citrate, pH = 7.0

Electrolyte buffer:

Tris.	16.35 gm.
Citric acid	9.04 gm.

Gel buffer:

Dilute electrolyte	15X

(Shaw and Prasad, 1970; their number I)

II Discontinuous Tris./citrate, pH = 8.6

Electrolyte buffer:

Boric acid	18.5 gm.
IN Sodium hydroxide	50ml.

Gel buffer:

Tris.	9.2 gm.
Citric acid	1.05 gm.

(Poulik, 1957).

III Lithium/Borate, pH = 8.5.

Electrolyte buffer:

Lithium hydroxide	2.52 gm.
Boric acid	14.15 gm.

Gel buffer;

Tris.	9.57 gm.
Citric acid	1.47 gm.
Electrolyte buffer	156 ml.

(Gahne, 1966).

Electrophoresis with these buffers was normally carried out with a voltage drop of about 120-150 volts across the ends of the gel, and a current of about 40 milliamps per gel. Most runs lasted approximately four hours.

After electrophoresis gels were sliced horizontally into two or three slices with a steel guitar string, and placed, cut surface up, in a stainless steel tray for staining.

3.5. Gel staining methods

The specific procedures used for each enzyme are given below, in alphabetical order. This includes the buffer system used for electrophoresis, whether starch or polyacrylamide was used, and the ingredients and conditions used for staining. All quantities in recipes are for staining 32 specimens.

Several different buffers were used for staining:-

IV 0.5M Tris./HCl, pH = 7.5.

Tris.	60.57 gm.
1N HCl	414 ml.

V 0.5M Tris./HCl, pH = 8.5.

Tris	60.57 gm.
1N HCl	165 ml.

VI 0.1M Tris./maleate, pH = 6.5.

Tris.	12.1 gm.
Maleic acid	11.6 gm.
1N Sodium hydroxide	84 ml.

VII Acetate, pH = 5.4.

Glacial acetic acid	6.8 ml.
Sodium hydroxide	4 gm.

Unless otherwise stated, gels were stained with the aid of a mechanical shaker.

Aldehyde Oxidase

Electrophoresis in buffer system I, on polyacrylamide.

Stain:

Benzaldehyde	2 ml.
MTT	50 mg.
PMS	5 mg.
Stain buffer IV	100 ml.

Incubate at room temperature for a few minutes, until blue bands appear. De-stain in 10% acetic acid.

Amylase

Electrophoresis in buffer system III, on starch.

Stain:

p-phenylenediannine dihydrochloride	2 gm.
Stain buffer VII	200 ml.

Cover and incubate at 37°C for several hours, without shaking. Amylase bands become visible as pale zones against a dark blue/black background. Continue to incubate at 37°C overnight. Remove stain and soak in 5:5:1 methanol:water:acetic acid for three hours followed by distilled water for two hours. Amylase bands are then visible as transparent zones in an otherwise opaque gel.

Esterase

Electrophoresis in buffer system III, on starch.

Stain:

α-naphthyl acetate	50 mg.
β-naphthyl acetate	50 mg.
(pre-dissolved in a few ml. of acetone)	
Fast Garnet GBC salt	50 mg.
0.05M $CaCl_2$	10 ml.
Stain buffer VI	100 ml.
Methanol	100 ml.

Incubate at room temperature. Red/purple bands become visible in a few minutes and reach maximum intensity within an hour. De-stain in 5:5:1 methanol:water:acetic acid.

General protein

Electrophoresis in buffer system I, on polyacrylamide.

Stain:

0.05% Coomassie Blue	100 ml.
(in 10% Trichloroacetic acid).	

Incubate at room temperature overnight.

De-stain for several hours in 10% Trichloroacetic acid.

General protein could also be scored on starch in almost any electrophoresis buffer. In this case the stain was 1% Amido Black, in 5:5:1 methanol:water:acetic acid, and the gel was de-stained in several changes of this solution.

Glucose-6-phosphate dehydrogenase

Electrophorsis in buffer system I, on polyacrylamide.

Stain:

Glucose-6-phosphate, disodium salt	100 mg.
NADP	20 mg.
MTT	50 mg.
PMS	10 mg.
Na_2 EDTA	100 mg.
Stain buffer IV	100 ml.

Incubate at room temperature for several hours until blue bands appear. De-stain in 10% acetic acid.

Could be stained on the same slice of gel as Aldehyde oxidase.

Glutamate dehydrogenase

Electrophoresis in buffer system II, on starch.

NAD added to gel and electrolyte at the rate of 40 mg./litre.

Stain:

L-glutamic acid, sodium salt	1 gm.
MTT	50 mg.
PMS	10 mg.
NAD	40 mg.
Stain buffer IV	100 ml.

Incubate at room temperature, in the dark, for three hours or until blue bands appear. De-stain in 5:5:1 methanol:water:acetic acid.

Lactate dehydrogenase

Electrophoresis in buffer system I, on polyacrylamide.

Stain:

70% Sodium lactate solution	2 ml.
NAD	40 mg.
MTT	50 mg.
PMS	10 mg.
Stain buffer IV	100 ml.

Incubate at room temperature in the dark, for several hours or until blue bands appear. De-stain in 10% acetic acid.

Could be stained on the same slice of gel as Malate dehydrogenase.

Leucine aminopeptidase

Electrophoresis in buffer system I, on polyacrylamide.

Stain:

L-leucyl β-naphthylamide (pre-dissolved in a few ml. of acetone)	50 mg.
Fast Garnet GBC salt	50 mg.
Stain buffer VI	100 ml.

Incubate overnight at room temperature. Activity appears as orange zones on a yellow background. De-stain in 10% acetic acid.

Malate dehydrogenase

Electrophoresis in buffer system I, on polyacrylamide.

Stain:

L-malic acid	100 mg.
NAD	40 mg.
MTT	50 mg.
PMS	10 mg.
Stain buffer IV	100 ml.

Incubate in the dark at room temperature for one hour or until blue bands appear. Stain both cathodal and anodal ends of the gel. De-stain in 10% acetic acid.

Malic enzyme

Electrophoresis in buffer system I, on polyacrylamide.

Stain:

L-malic acid	100 mg.
NADP	25 mg.
MTT	50 mg.
PMS	10 mg.
$MgCl_2$	100 mg.
Stain buffer IV	100 ml.

Incubate in the dark at room temperature for one hour or until blue bands appear. De-stain in 10% acetic acid.

Superoxide dismutase

Electrophoresis in buffer system II, on starch.

Stain:

MTT	50 mg.
PMS	10 mg.
Stain buffer V	100 ml.

Incubate at 37°C, illuminated, until white zones of activity appear against the blue background of the gel (several hours).

Whatever the staining system used, gels almost always contained specimens from at least three nests, and usually of at least two colour forms of ants, arranged across the gel (16 slots) in arbitrary order. After staining, gels were scored without knowledge of the identity of the specimens, and were usually scored independently several times. In any cases of doubt, the particular specimen or others from the same nest were re-run with varying combinations of other specimens.

3.6. Chromosome methods.

The method used for making chromosome preparations is that of Imai *et al.*, (1977; also Imai and Crozier, unpublished).

Immature ants were obtained from nests by excavation, and kept with workers in a darkened cage until they reached the correct stage. For male pupae, this was when the compound eyes reached a pale red colour, and for workers and queens the pre-pupal stage was used. The cerebral ganglia or testes were dissected out using mounted minuten pins, and placed on a welled slide, in two drops of 1% sodium citrate solution containing 0.005% colchicine. This was then left to stand for 10-20 minutes in a humid box at room temperature. The organ was then removed to a clean microscope slide and the citrate solution drained off. The slide was inclined at about 20^{o} and several drops of fixative 1 added to the organ (glacial acetic acid:absolute ethanol: water = 3:3:4). The organ was quickly macerated in a fresh drop of this solution, and a drop of fixative 2 was added (glacial acetic acid: absolute ethanol = 1:1). After 30 seconds these solutions were removed by inclining the slide laterally onto a sponge, and two drops of glacial acetic acid were added. After ten seconds the slide was again inclined to remove the fixative, and set aside to dry.

After at least one day, slides were stained for ten minutes in 10% Giemsa stain (Gurr's) in Sorenson's buffer (Na_2HPO_4, 4.75 gm; KH_2PO_4, 4.54 gms,; water to 1 litre, pH = 6.8). After staining the slides were washed for one second in running water and allowed to drain. Cover slips were mounted with Xam (Gurr's).

CHAPTER 4

POLYMORPHISM AT THE *AMYLASE* LOCUS

4.1. The phenotypes and their inheritance

Figure 4.1 shows photographs of two gels stained for amylase activity after electrophoresis of meat ant homogenates. Each individual has either one or two bands of activity, which appear to correspond to homozygous and heterozygous genotypes respectively. The observed phenotypes can be accounted for by the existence of four co-dominant alleles at one locus. The locus is designated *Amylase (Amy)*, and the four alleles Amy^a, Amy^b, Amy^c, Amy^d, in decreasing order of anodal mobility. The interpretation of the phenotypes in terms of these alleles is shown on the Figure. This model predicts that the maximum number of bands of activity in an individual should be two unless heterozygotes have extra zones resulting from dimeric (or larger) quarternary structure of the enzyme. In the thousands of workers which were run, no more than two bands of amylase activity were found in any specimen. Multiple banded phenotypes could be produced artificially however, by the mixing of extracts from different individuals. Combinations tried included Amy^aAmy^a with Amy^bAmy^d, Amy^aAmy^c with Amy^bAmy^d, Amy^aAmy^c with Amy^dAmy^d, and Amy^aAmy^a with Amy^dAmy^d. In each case the resulting phenotype was the simple sum of the phenotypes of the individuals used in the mixture.

Staining for amylase on gels containing starch may produce spurious mobility variation, caused by differences in the concentration of individual extracts (Boettcher and de la Lande, 1970). The slight variation in the mobility of the amylase allozymes evident in Figure 4.1 may be attributed to this cause, or to inconsistency in the quality of the gel. If these differences in mobility reflect heterogeneity within the "alleles" concerned (Bernstein *et al.*, 1973), the result will be a loss of information, rather than the creation of false in-

Figure 4.1.

Photographs of gels stained for *Amylase* activity. O = origin.

Direction of migration upward.

Slightly larger than actual size.

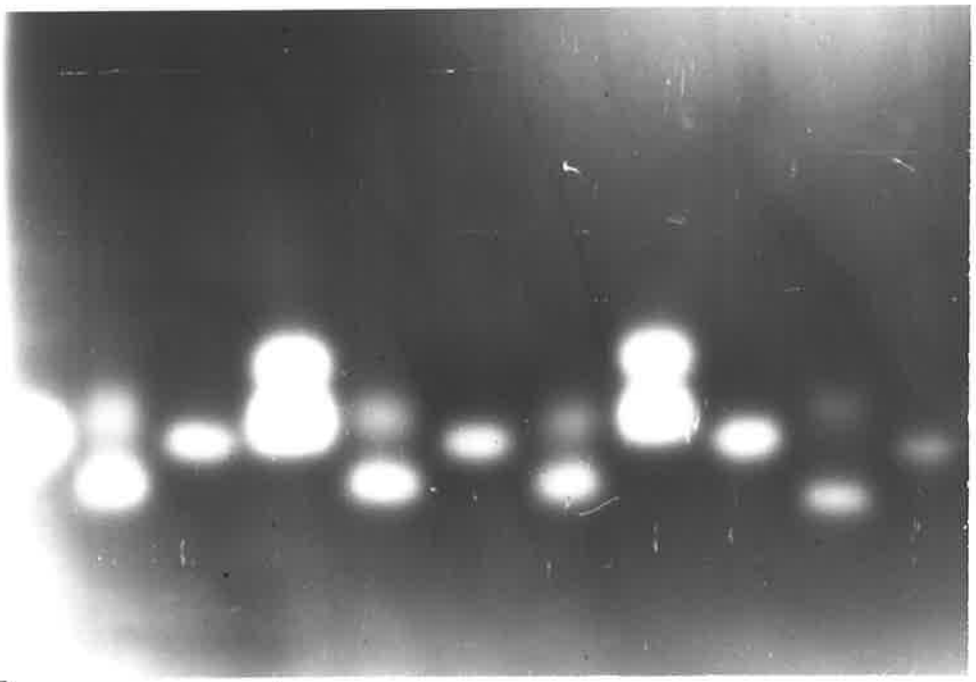
Genotypes indicated in abbreviated notation

a = Amy^a

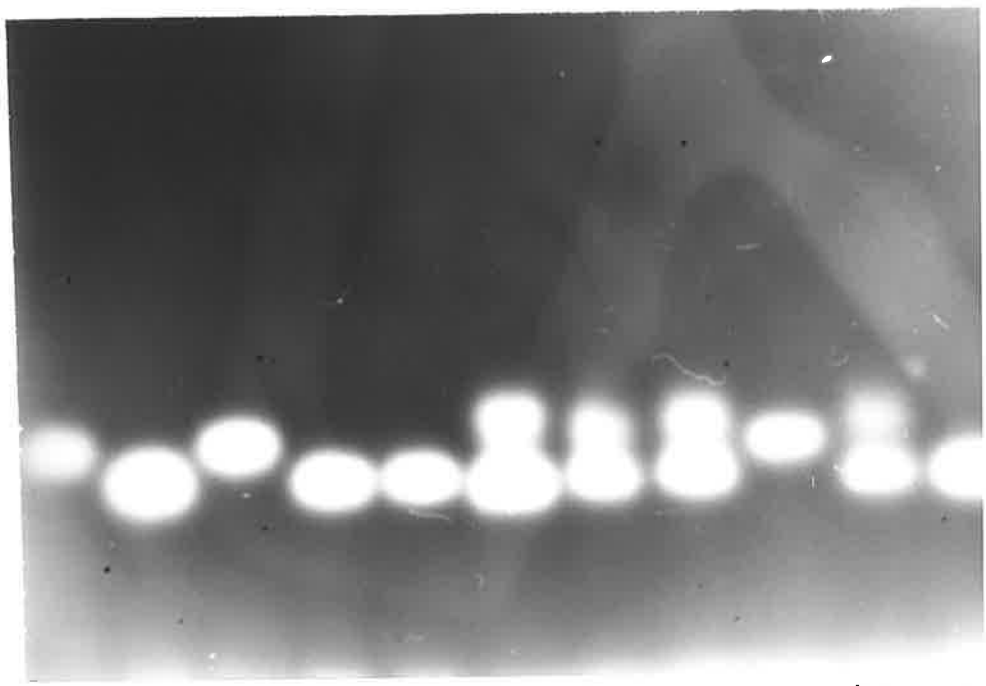
b = Amy^b

c = Amy^c

d = Amy^d



O– cc bd cc ac bd cc bd ac cc bd cc



O – cc dd cc dd dd bd bd bd cc bd dd

formation, so any error involved will be in the direction of conservatism.

Controlled matings to confirm the genetic basis of phenotypes are not yet possible in meat ants. A number of attempts were made to induce or force mating in captivity based on the methods used for ants (Cupp *et al.*, 1973), bees (Kerr and Laidlaw, 1956), and mosquitoes (McDaniel and Horsfall, 1957), but they were not successful. However, since nests are family units, some evidence can be obtained from the patterns of segregation found within nests.

If there is one singly mated queen per nest, any nests which segregate should show a 1:1 ratio of homozygous:heterozygous workers. There should be either one or two genotypes among the workers from a particular nest, and the isozyme in homozygotes should have the mobility of one of those in the heterozygotes. A total of 76 nests segregated for *Amylase*, and the results from 20 chosen at random from among these are shown in Table 4.1. For these purposes, the number of workers of the same genotype sampled from a nest classified as "non-segregating" was almost always seven or more, so it seems likely that very few segregating nests were missed.

The data in Table 4.1 reveal several interesting points. Firstly, almost all the results can easily be accounted for by the presence of one singly mated queen per nest, if the *Amylase* phenotypes are produced by four co-dominant alleles at one locus. Secondly, in most cases, it is a simple matter to work out the genotypes of the queen and male which founded the nest. For example, nest 113 contained 6 $Amy^{d}Amy^{d}$ workers and 9 $Amy^{b}Amy^{d}$. This is consistent with expectations from the mating $Amy^{b}Amy^{d} \times Amy^{d}$. The third point is that some nests do not fit this simple pattern. Nest 394 shows a 4:15 ratio, which is significantly different from the 1:1 expectations ($\chi^2_1 = 6.37$, $P = 0.01\text{-}0.02$). Nests 287 and 406 each contain three genotypes, so they cannot be accounted

Table 4.1. *Amylase* genotypes in workers from 20 randomly selected segregating nests.

Allele notation abbreviated:

i.e., a = Amy^a *etc.*

Colour forms:

P = red form
B = blue form
V = black form

Nest number	Form	Worker Genotypes
28	P	7cc : 3ac
56	P	6cc : 4ac
113	B	6dd : 9bd
119	P	3cc :10ac
127	B	3dd : 5bd
144	V	5cc : 3cd
279	B	9bb :10bd
285	B	12dd: 10bd
287	B	4dd : 8bd : 6bb
379	B	5dd : 3bd
387	B	4bb : 8bd
394	B	4bb :15bd
402	B	3dd : 5bd
406	B	6dd : 7bd : 2bb
410	B	7dd : 3bd
427	B	3bb : 5bd
458	P	5cc : 3ac
462	B	3dd : 5bd
467	B	4dd : 4bd
477	B	3bb : 5bd

for by the simple model proposed. This gives strong evidence for the presence of more than one queen in some nests ("polygyny").

Further evidence on the mode of inheritance of the *Amylase* phenotypes can be obtained by typing males and workers from the same nests. Males were not easy to obtain, and their *Amylase* activity was found to be weak, so some gels did not yield any results. The results for the nests where both males and workers could be scored are shown in Table 4.2. Although many of these nests are not informative, the results are all consistent with the genetic model proposed. They are also consistent with the suggestion that males are produced from unfertilized eggs laid by the queen (see Chapter 8).

Circumstantial evidence can also be obtained from the similarity between the *Amylase* variation described here, and that recorded for other species. Phenotypes similar to those shown in Figure 4.1 have now been observed in many species of animals and plants, and for a wide range of enzymes and proteins. In those cases where a rigorous genetic analysis has been made, these phenotypes have almost invariably been found to be controlled by co-dominant alleles at a single locus (Powell, 1975).

For this reason, and from the data presented in Tables 4.1 and 4.2, it can be claimed with some confidence that the *Amylase* variation in meat ants is controlled by four co-dominant alleles at one locus.

Table 4.2. *Amylase* genotypes of workers and males in all nests where both have been scored.

Abbreviations as in Table 4.1.

Nest	Form	Workers	Males	Inferred Mating
31	B	13dd : 7bd	3d : 2b	bd x d
54	P	8cc	7c	cc x c
98	P	6cc	6c	cc x c
131	P	7cc	8c	cc x c
134	P	22ac	6c	cc x a
201	P	20cc	2c	cc x c
212	P	6cc : 4ac	2a : 6c	ac x c
228	P	9cc	8c	cc x c
234	P	23cc : 5cd	13c : 7d	cd x c
235	P	11cc	11c	cc x c
236	P	8cc	8c	cc x c
237	P	8cc	5c	cc x c
408	P	8cc	8c	cc x c
410	B	7dd : 3bd	2d	bd x d
411	B	5dd : 3bd	1d	bd x d
418	P	18ac	8c	cc x a
425	P	12cc	3c	cc x c
455	P	10cc	8c	cc x c
456	P	10cc	8c	cc x c

4.2. Estimation of gene frequencies

If this genetic model is correct, it should be a simple matter to estimate the frequencies of the alleles in populations. The procedure is to score a series of workers from a nest, use their genotypes to obtain the genotypes of the founding queen and male, and derive gene frequencies by direct gene counting among these reproductive individuals. For example, the population of the red form from Arthurton comprises 18 nests with workers all $Amy^{C}Amy^{C}$, and a single nest with $8Amy^{C}Amy^{d}$: $8Amy^{C}Amy^{C}$. This nest presumably came from the mating $Amy^{C}Amy^{d} \times Amy^{C}$, so the gene frequency of Amy^{d} is 1/57 = 0.0175, based on the supposition that each nest contributes three alleles to the count. However, this simple procedure is subject to a number of problems.

a) Some genotypes cannot be scored unambiguously.

A nest containing workers which are all $Amy^{a}Amy^{C}$ could have been produced by either of the matings $Amy^{a}Amy^{a} \times Amy^{C}$ or $Amy^{C}Amy^{C} \times Amy^{a}$, so in these nests, the genotypes of the founders cannot be clearly identified. There are a number of ways of solving or avoiding this problem.

One approach is to ignore these nests completely. Contel and Mestriner (1974) used this procedure when estimating esterase allele frequencies in the social bee *Melipona subnitida*. One step further from this is to estimate the frequency in those nests which can be scored unambiguously, and use this result to aid in the scoring of the ambiguous nests. A Barossa Valley population of the red form of meat ants contains 11 nests with workers all $Amy^{C}Amy^{C}$, one nest segregating $7Amy^{C}Amy^{C}:3Amy^{a}Amy^{C}$, and one nest containing $9Amy^{a}Amy^{C}$. Excluding this last nest, the gene frequency of Amy^{a} is 1/36 = 0.0278. Therefore, the mating $Amy^{C}Amy^{C} \times Amy^{a}$ will occur in this population much more often than $Amy^{a}Amy^{a} \times Amy^{C}$, and the ambiguous nest has probably been produced

by the former mating. The revised gene frequency for *Amy*^{a} is then 2/39 = 0.0513. This approach has the unfortunate feature of being circular, and is of little use when gene frequencies are approximately equal. However, when used in a population such as this one, the error involved must be slight.

Crozier (1973a, 1977b) has developed an iteration procedure for classifying the ambiguous nests at the *Mdh-a* locus in the ant *Aphaenogaster rudis*. It relies on the fact that the frequencies of some of the types of unambiguous nests are related to the gene frequencies in a simple way. If the gene frequency of *Mdh-a*^{2} is q then the frequency (c) of nests segregating *Mdh-a*^{1}*Mdh-a*^{2}*:Mdh-a*^{1}*Mdh-a*^{1} is $2q(1-q)^2$, produced by the mating *Mdh-a*^{1}*Mdh-a*^{2} *x Mdh-a*^{1}. Also, the frequency (d) of nests which segregate *Mdh-a*^{1}*Mdh-a*^{2}*:Mdh-a*^{2}*Mdh-a*^{2}, is $2q^2(1-q)$. The ratio c/(c+d) then gives a first approximation to q. This estimate of the gene frequency can then be used to partition the ambiguous class of nests into its two components, and a new gene frequency can be calculated from the results. This process is then repeated until the change in successive estimates of the gene frequency is sufficiently close to zero.

Of course, these problems can be avoided completely if males can be collected and scored from the ambiguous nest. If males are produced from unfertilized eggs laid by the queen (rather than workers), they should give a clear indication of her genotype. The use of males in this way has been possible in some of the meat ant nests considered here (*e.g.*, nest 418 in Table 4.2).

Where the genotype of the queen cannot be obtained in this way, yet another procedure can be used. In a population with two alleles, say *Amy*^{b} and *Amy*^{d}, there are six possible matings, produced by three genotypes in females and two in males. With random mating and Hardy-Weinberg equilibrium, the expected frequencies of these matings can be

derived as functions of the gene frequencies (see Table 4.3). Five different types of nest result, and the expected and observed frequencies of these can be used to estimate gene frequencies by use of the method of maximum likelihood. This process is described more fully in the context of the *Esterase-1* locus in the next chapter.

b) Populations may contain more than two alleles.

Almost all the meat ant populations examined have only two *Amylase* alleles. The only exception is the Flinders Ranges population of the blue form. Here, all the nests contain only Amy^b and Amy^d, except for one which contains $7Amy^cAmy^d : 8Amy^dAmy^d$ workers. In this case, the alleles Amy^b and Amy^c were pooled in the maximum likelihood analysis, to give a combined frequency for $Amy^{(b+c)}$ of 0.500. The actual frequency of Amy^c in these 11 nests must be 1/33 = 0.030, so the frequency of Amy^b is 0.470, and that of Amy^d is 0.500.

c) Some nests contain more than one queen.

The results in some segregating nests indicate that they do not conform to the rule of one singly-mated queen per nest. Nest 406, in the Dublin population of the blue form, contains $6Amy^dAmy^d : 7Amy^bAmy^d : 2Amy^bAmy^b$ workers. The occurrence of three phenotypes in this way gives clear evidence of either the presence of more than one queen in the nest ("polygyny"), or of multiple matings of a single queen. In many cases it is not possible to distinguish between these two alternatives, but polygyny seems more likely than multiple mating. These nests have therefore been considered as the result of two matings instead of one. Nest 406 is treated as the combination of the matings $Amy^bAmy^d \times Amy^b$ and $Amy^bAmy^d \times Amy^d$, and contributes six genes to the total instead of three. The alternative is to consider this nest as the product of a single Amy^bAmy^d queen and two males, but the difference in the resulting gene frequencies is very slight. More serious problems are caused

Table 4.3. The six possible matings and their expected frequencies for a locus with two co-dominant alleles.

Allele notation abbreviated as before. The gene frequency of *Amg*^{b} is q.

Mating	Frequency	Workers
bb x b	q^3	All bb
bd x b	$2q^2 (1 - q)$	½bb : ½bd
bd x d	$2q (1 - q)^2$	½dd : ½bd
dd x d	$(1 - q)^3$	All dd
bb x d	$q^2 (1 - q)$	All bd
dd x b	$q (1 - q)^2$	All bd

by the fact that polygyny can be detected only in a minority of cases, where the correct combination of genotypes occurs. Nests which do not segregate give no information on this point, and polygyny in them goes undetected. Thus, one effect of this approach is to inflate the frequency of the less common allele. Also, polygyny in some cases may not result in the appearance of a third genotype, but only in a disturbance of the segregation ratio, so it cannot always be definitely identified.

When estimating gene frequencies, therefore, I have counted nests twice only where there is clear evidence of polygyny, either through the existence of three genotypes or a grossly disturbed segregation ratio. Although this does involve some inaccuracy, it is the best that can be done at the moment. The effects of polygyny are considered in more detail in Chapter 8.

As shown by the examples used, the problems of ambiguous nests, multiple alleles, and polygyny, have been approached in various ways in different populations. Nevertheless, all the methods used aim at obtaining the gene frequency in the reproductive part of the population. Others have estimate gene frequencies for a locus with co-dominant alleles in social Hymenoptera directly from workers (Mestriner and Contel, 1972; Johnson *et al.*, 1969; Tomazewski *et al.*, 1973; Martins *et al.*, 1977). Either four or five workers were typed from each nest, and these contributed either eight or ten genes to the sample. This procedure gives an artificially inflated estimate of the number of genes actually sampled. For example, Tomazewski *et al.*, (1973) typed five workers of the ant *Pogonomyrmex badius* from each of about 15 nests per population, to obtain a mean sample size of about 70 individuals, or 140 genes. However, if only the parents are considered, nests should actually contribute only 45 genes to the sample. Mestriner and Contel

(1972) typed four workers from each of 75 hives of honeybees, for a total of 300 bees, or 600 genes. These hives should only contribute 225 genes to the sample.

The direct use of workers may influence the gene frequency estimate itself, as well as the sample size. In a meat ant nest segregating 1:1 for, say, $Amy^{C}Amy^{C}$ and $Amy^{a}Amy^{C}$ workers, a sample of five workers will contribute, on average, 7.5 Amy^{C} and 2.5 Amy^{a} genes to the sample. However, the queen and male contribute two Amy^{C} genes and one Amy^{a}. The direct use of workers will therefore make rare alleles appear even rarer than they really are. In the Barossa Valley population of the red form of meat ants, there are 13 nests, giving 39 genes in males and females. The estimated gene frequency of Amy^{a} in these individuala is 0.051. Using the first five workers from each nest gives 130 genes, and a frequency for Amy^{a} of 0.046. The direct use of workers for the estimation of gene frequencies is therefore to be avoided, despite the reduction in labour, and the apparent increase in sample sizes which it allows.

The *Amylase* allele frequencies among reproductives in 23 populations of meat ants are shown in Table 4.4. Out of the 23 populations, most are too small to test for Hardy-Weinberg equilibrium, or show little or no polymorphism. The eight which can be tested are marked with an asterisk in the table, and all show good agreement with expectations. Also, these eight populations show no significant differences in gene frequency between males and females.

Once gene frequencies have been obtained, they can be used for the comparison of populations, both within and between colour forms.

Table 4.4. *Amylase* allele frequencies in meat ant populations. For localities see Figure 3.1.

*Populations which can be tested for Hardy-Weinberg equilibrium.

Population	Amy^a	Amy^b	Amy^c	Amy^d	No. of genes in sample.
Red form :-					
Barossa Valley	0.051	0	0.949	0	39
Eyre Penin.	0.143	0	0.857	0	42 *
Arthurton	0	0	0.982	0.018	57
Dublin	0	0	0.980	0.020	51
Eudunda	0.111	0	0.889	0	63 *
Morgan	0.233	0	0.767	0	60 *
Adelaide	0	0	1.000	0	51
Flinders Ra.	0.200	0	0.800	0	15
Southeast	0.067	0	0.933	0	15
Upper Murray	0.083	0	0.917	0	12
Blue form :-					
Hallett Cove	0	0.417	0	0.583	12
Eyre Penin.	0	0.280	0	0.720	27 *
Flinders Ra.	0	0.470	0.030	0.500	33 *
Dublin	0	0.558	0	0.442	54 *
Eudunda	0	0.471	0	0.529	54 *
Morgan	0	0.509	0	0.491	57 *
Snowtown	0	0.333	0	0.667	15
Black form :-					
Flinders Ra.	0	0	0.952	0.048	21
Eyre Penin.	0	0	0.944	0.056	18
Morgan	0	0	1.000	0	45
Yellow form :-					
Eyre Penin.	0	0	1.000	0	30
Small purple form :-					
Eyre Penin.	0	0.067	0	0.933	15
Mundoora	0	0	0	1.000	12

4.3. Geographic variation within forms.

The small amount of data described previously (Halliday, 1975) did not allow the detection of any geographic variation of *Amylase* allele frequencies within colour forms. When populations are sampled separately, this does become possible, and the results for 10 populations of the red form are presented in Figure 4.2. All these populations share the common allele Amy^c, which is fixed in the Adelaide population. Amy^a reaches a maximum of 0.233 at Morgan, and Amy^d is represented by a single occurrence in each of the Dublin and Arthurton populations.

The populations of the red form show significant heterogeneity with respect to *Amylase* allele frequencies ($2 \times 10\ \chi^2_9 = 32$, $P<0.001$). For the purposes of this test the frequencies of Amy^a, Amy^b, and Amy^d were pooled, and jointly compared with Amy^c. In general, populations which are close geographically also have similar *Amylase* allele frequencies, although this relationship is not a simple one. Complications are introduced by the fact that the red form has a somewhat fragmented geographic distribution in South Australia, which may contribute to the geographic variation observed for allele frequencies.

For the blue form, *Amylase* allele frequencies are shown in Table 4.4 and Figure 4.3. All populations have Amy^b and Amy^d in moderate frequencies, with the frequency of Amy^b ranging from 0.280 on Eyre Peninsula to 0.558 at Dublin. A single occurrence of Amy^c in the Flinders Ranges gives it a frequency of 0.030 in that population. Most of the allele frequencies for *Amylase* in the blue form were estimated by maximum likelihood. The differences between populations were therefore tested by χ^2 tests of heterogeneity among nest type numbers, rather than among counts of alleles, since the latter could not be

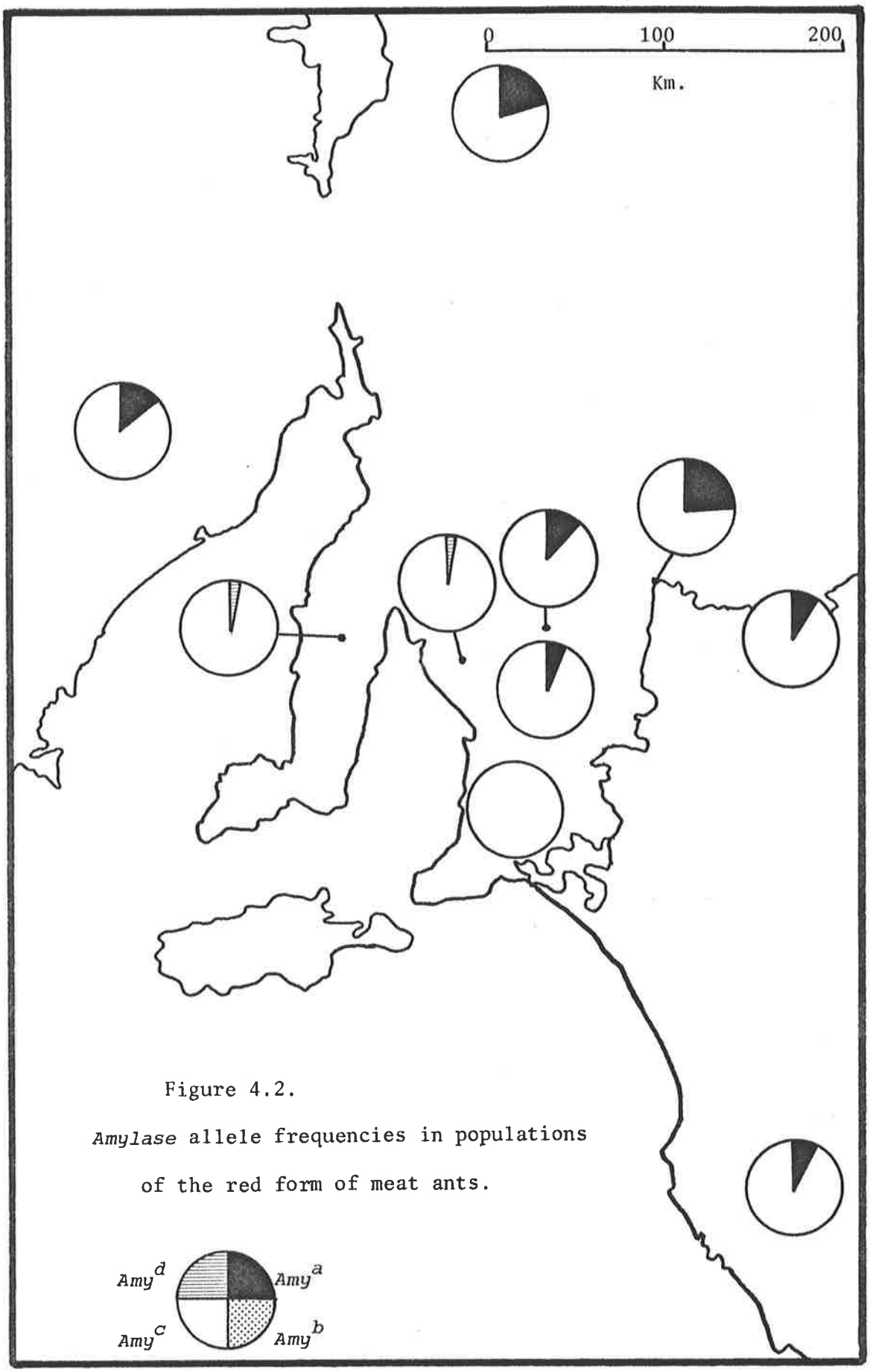


Figure 4.2.

Amylase allele frequencies in populations of the red form of meat ants.

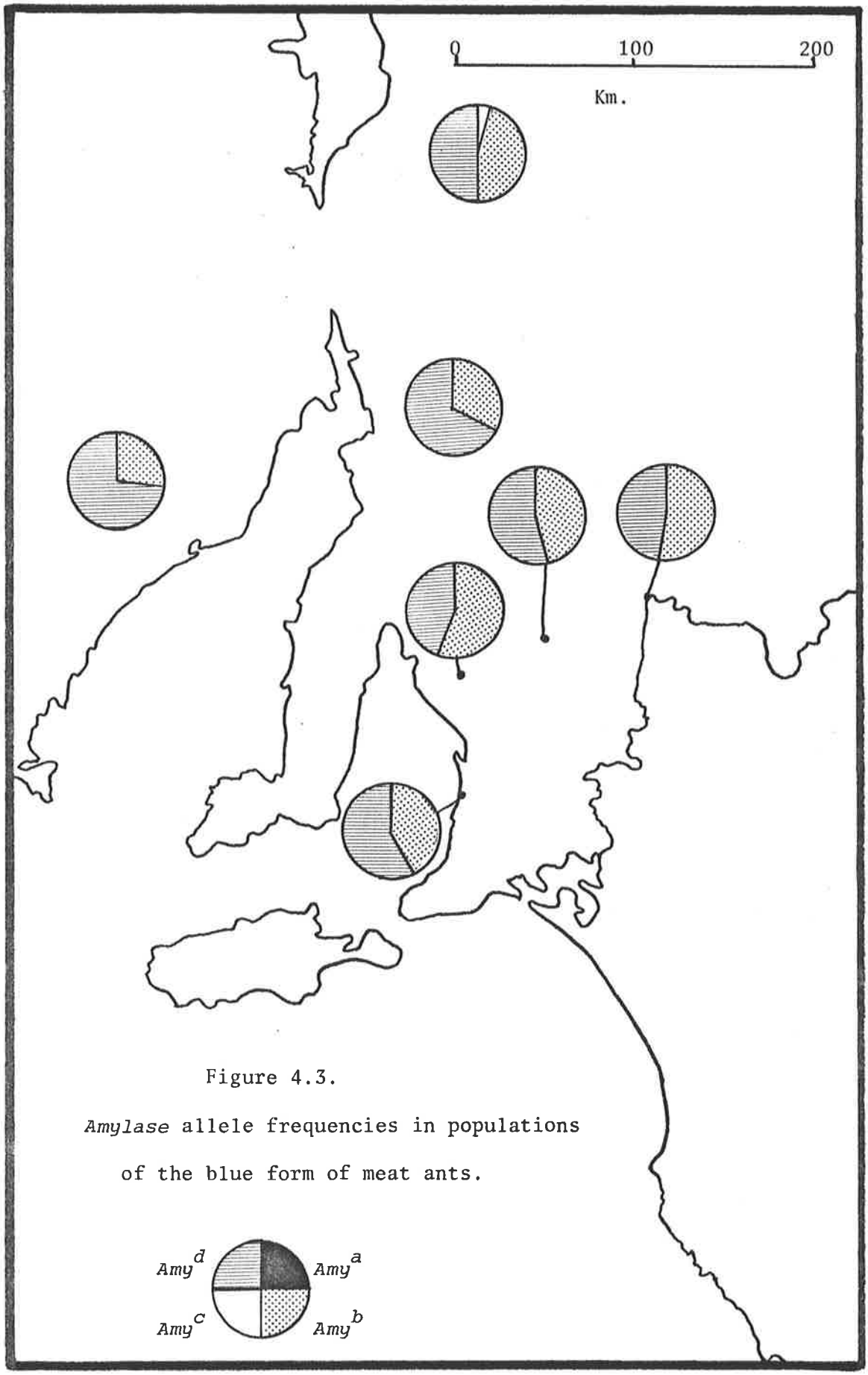


Figure 4.3.

Amylase allele frequencies in populations of the blue form of meat ants.

directly obtained. It was necessary to pool nests segregating $Amy^{b}Amy^{b}$: $Amy^{b}Amy^{d}$ with those containing all $Amy^{b}Amy^{b}$, and also to pool nests segregating $Amy^{d}Amy^{d}$:$Amy^{b}Amy^{d}$ with those containing all $Amy^{d}Amy^{d}$. Further, the Hallett Cove and Snowtown populations could not be included due to their small sample sizes. The resulting three nest types in five populations yield a heterogeneity χ^2_8 of 9.83, which does not indicate significant heterogeneity on the basis of these data.

As in the case of red form, some of the areas where the blue form was collected are separated by either physical barriers or populations of other colour forms, which tend to fragment its distribution to some extent. However, for this form, this does not seem to be reflected by heterogeneity in *Amylase* allele frequencies. The data for other colour forms are insufficient to give any indication of whether they also vary geographically.

The factors contributing to geographic variation in *Amylase* allele frequencies are considered more fully in Chapter 7, after the results from other isozyme loci have been presented in Chapters 5 and 6.

4.4. Differences among forms.

Table 4.4 shows that several opportunities exist for comparing the *Amylase* allele frequencies of two or more different colour forms in areas where they are sympatric. The red and blue forms are sympatric or very close together at Morgan, Dublin, Eudunda, and Eyre Peninsula. Also, the Hallett Cove blue form population is surrounded by the Adelaide red form population. In all these areas they have almost no alleles in common, which is strong evidence that they are not interbreeding.

Exceptions are the occurrence of Amy^d in the red form at Arthurton and Dublin, and Amy^c in the blue form in the Flinders Ranges. At the moment it is not possible to determine whether these represent the results of recent hybridization, or the permanent presence of these alleles at low frequency.

At Morgan, the black form appears to be fixed for Amy^c, so it is also very different from the sympatric blue form. Comparing the black and red forms at Morgan also reveals a significant difference in allelic composition (Fisher's exact 2 x 2, with pooling of Amy^a with Amy^d, P = 0.02), so all three forms seem to be reproductively isolated from each other in this area.

Many more comparisons are possible among the Eyre Peninsula collections, involving five colour forms. Some pairs of colour forms have almost no alleles in common, and for others, the difference in their allele frequencies has been tested, using Fisher's exact 2 x 2 test. The results are shown in Table 4.5. The differences between the frequencies for the yellow and black forms, and the blue and small purple forms, are not significant at the 0.05 level, while the comparison of the red and yellow forms, is marginally significant and probably not reliable. For the comparison of the red and black forms,

Table 4.5. Comparison of *Amylase* allele frequencies among five colour forms of meat ants from Eyre Peninsula.

P = red form
V = black form
Y = yellow form
B = blue form
SP = small purple form

n.s. = not significantly different (in the marginal case, the probability level is shown)

* = the forms being compared have almost no shared alleles.

	P	V	Y	B	SP
P	-				
V	n.s.	-			
Y	0.034	n.s.	-		
B	*	*	*	-	
SP	*	*	*	n.s.	-

the exact 2 x 2 test requires pooling of Amy^a and Amy^d frequencies, for comparison with Amy^c. This results in a probability of 0.244. Therefore it is not possible to obtain evidence of reproductive isolation between those forms with these data, despite the fact that they contain different alleles. All other comparisons show large differences in allele frequency between pairs of colour forms.

This is consistent with the previous results (Halliday, 1974, 1975) which indicated reproductive isolation between some colour forms. The differences are especially marked between those forms which build large multi-entrance mound nests (P, V, Y) and those which do not, at least in South Australia (B, SP).

The fact that many colour forms have distinctive *Amylase* allele frequencies makes it possible to use this locus to identify some types of specimens. The Snowtown population described earlier comprises five nests of the blue form, which are surrounded by populations of the red form. In a mapping survey (Greenslade, 1976b) it was found that these nests contained some very pale specimens which approached the colour of some specimens of the red form, as well as many typical dark blue workers. This suggested the possibility of hybridization between these forms. However, all five nests from this population contained the typical blue form *Amylase* alleles, with Amy^b and Amy^d both present in moderate frequencies. A single red form nest 400 metres away was sampled, and contained 8 Amy^aAmy^a and 7 Amy^aAmy^c workers. These results give no evidence of any introgression of genes from the red form into the blue form.

A second example of the usefulness of this locus for separating colour forms concerns the Mundoora population of the small purple form. When first discovered it was not known whether this population was genuinely of the small purple form, or an aberrent isolate of

either the red or blue form. The four nests sampled contained only *Amy*^{d}*Amy*^{d} workers (nine or ten from each nest). Since populations of the red form rarely contain this gene at all, and the blue form normally has the allele *Amy*^{b} at a frequency of at least 0.3, it is very unlikely that this population belongs to either of these forms. It most resembles the Eyre Peninsula population of the small purple form, which has a gene frequency for *Amy*^{d} of 0.933. This makes it clear that the Mundoora population is genuinely of the small purple form, as suggested by its appearance and nest structure.

The *Amylase* locus provides clear evidence of reproductive isolation between the following pairs of colour forms of meat ants:-

Red - Blue

Red - Small Purple

Black - Blue

Black - Small Purple

Yellow - Blue

Yellow - Small Purple.

There is also some indication of a lack of gene flow between the yellow and red forms. These conclusions would be strengthened if similar results could be obtained from other loci. Further discussions of these results is therefore postponed (Chapter 7) until evidence from other loci has been presented (Chapters 5 and 6).

CHAPTER 5

POLYMORPHISM AT THE *ESTERASE-1* LOCUS.

5.1. The phenotypes and their inheritance

Staining gels, for non-specific esterase activity activity after electrophoresis reveals complex arrays of bands in many organisms - *e.g.*, ants (Johnson *et al.*, 1969), snails (Oxford, 1973), and humans (Harris and Hopkinson, 1976), and it is usually possible to identify the effects of several loci. Null alleles have often been found at these loci, especially in insects *e.g.*, Lepidoptera (Handford, 1973), Diptera (Zouros and Krimbas, 1969), and Hymenoptera (Crozier, 1977b). The detection of null alleles may rely on apparent deficiencies of individuals heterozygous for two active mobility alleles, since the null homozygote may be rare. Even when the null allele is relatively common, extensive crosses may be needed to confirm its existence (Burns and Johnson, 1967). It is not known to what extent lack of activity on an artificial substrate *in vitro* reflects a real lack of *in vivo* activity. This problem is exaggerated by the fact that very little is known about the real metabolic role of these so-called "esterases". Nevertheless, it is convenient for practical purposes to regard apparent lack of activity as indicating homozygosity for a null allele.

Figure 5.1 is a photograph of a gel stained for esterase after electrophoresis of meat ant extracts (see also Figures 6.2 and 6.3). Up to six bands of activity are visible in each individual. This chapter deals with the slowest of these bands (*i.e.*, the most cathodal). The variation in this band is of the presence/absence type, and there is rarely any doubt as to whether it is present in an individual or not This variation is attributed to the locus *Esterase-1 (Es-1)*. The other bands of esterase activity are attributed to the effects of genes at

Figure 5.1.

Photograph of a gel stained for esterase, showing variation produced by alleles at the *Esterase-1* locus.

Es-1 phenotypes are shown as + or - (null).

Es-1 = site of activity of the *Esterase-1* enzyme.

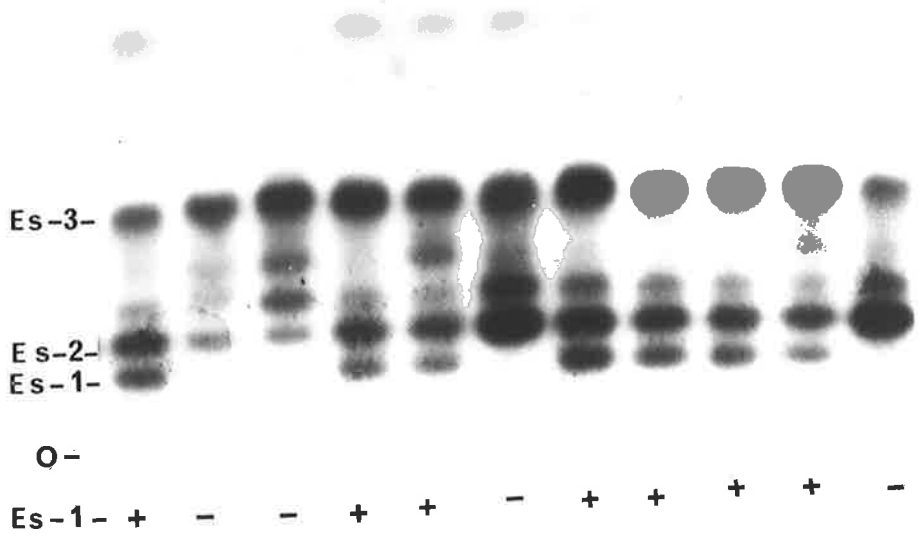
Es-2 = site of activity of the *Esterase-2* enzyme.

Es-3 = site of activity of the *Esterase-3* enzyme.

O = origin

Direction of migration upward.

Slightly larger than actual size.


Es-3-
Es-2-
Es-1-
O-
Es-1- + - - + + - + + + + -

other loci (*e.g.*, *Es-2* and *Es-3*), which are described in the next Chapter.

The bands of activity, and the loci producing them, are numbered unconventionally from the origin towards the anode. This is necessary because the most anodal bands are not clearly resolved, which makes it impossible to decide where to start numbering from the anodal end.

The most likely genetic basis for the *Es-1* phenotypes is that the apparent lack of enzyme activity is caused by homozygosity for a recessive null allele. This can be tested by examining the *Es-1* phenotypes of males (which are haploid). Table 5.1 shows results from all nests in which both workers and males have been scored, and the probable mating responsible for each. The results are consistent with the suggestion that the phenotypes are controlled by a recessive null allele $Es\text{-}1^{O}$ and an active allele $Es\text{-}1^{+}$.

An alternative, although less plausible hypothesis, is that the null phenotype is produced by a dominant inhibitor allele, so only $Es\text{-}1^{+}Es\text{-}1^{+}$ homozygotes have the + phenotype. This possibility is eliminated by the finding of null males in nests where the workers are all + (nests 98, 235, 408, 418).

A further possible explanation for the phenotypes is that there is actually mobility variation, involving the *Es-1* enzyme and some other band of activity. The most likely candidate is the band immediately towards the anode from the *Es-1* enzyme. In Chapter 6 I describe variation in this band, attributed to the locus *Esterase-2*, which eliminates the possibility of this type of variation.

Table 5.1. Meat ant nests in which both workers and males have been typed for *Esterase-1*.

Allele notation abbreviated;

o = *Es-1*^{o}

\+ = *Es-1*$^{+}$.

Nest number	Workers		Males		Inferred Mating
	+	Null	+	Null	
98	6	0	0	6	oo x +
131	3	8	3	5	+o x o
134	0	8	0	8	oo x o
201	7	13	1	1	+o x o
212	0	10	0	8	oo x o
228	0	8	0	8	oo x o
234	17	13	19	8	+o x o
235	11	0	5	6	+o x +
236	0	8	0	8	oo x o
237	0	8	0	5	oo x o
408	8	0	5	3	+o x +
418	17	0	2	6	+o x +
425	6	5	1	2	+o x o
455	10	0	8	0	+? x +
456	10	0	8	0	+? x +

5.2. Estimation of gene frequencies

As pointed out in the previous chapter, the estimation of gene frequencies in social Hymenoptera involves a number of problems. The major obstacles are that males and queens cannot always be obtained, so their genotypes must be inferred from those of workers. For the *Es-1* locus, a further complication is added by the fact that heterozygotes cannot be recognized. However, with respect to the *Es-1* phenotypes of workers, meat ant nests are of three kinds - those in which the workers are "all +", "all null", and "segregating". In practise, a minimum of six (usually more) workers of the same phenotype have been scored from a nest before it is classified as either "all +" or "all null".

If q is the frequency of the null allele *Es-1*^{O}, the expected frequencies of these three nest types can be derived as functions of q. These frequencies are shown in Table 5.2. Once these expected frequencies are obtained, they can be combined with observed frequencies in a population, and q can be estimated by maximum likelihood.

If a population is observed to contain J nests with workers "all null", K with segregating workers, and M with workers "all +", then the logarithm of the likelihood of a particular value of q is given by

$$L = \text{Constant} + J\log q^3 + K\log(2q^2 - 2q^3) + M\log(1-2q^2 + q^3) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where q is the frequency of the null allele *Es-1*^{O}.

Differentiating this, and simplifying, gives

$$\frac{dL}{dq} = \frac{3J}{q} + \frac{2K - 3Kq}{q - q^2} + \frac{3Mq^2 - 4Mq}{1 - 2q^2 + q^3} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The estimated value of q for a population is then obtained by inserting the observed values of J, K, and M, and solving equation (2) when dL/dq = 0. This process has been carried out by the computer program QEST, which evaluates dL/dq for 200 values of q between zero and one.

Table 5.2. Observed and expected frequencies of the three nest types produced by all possible matings at the *Esterase-1* locus in terms of q, the allele frequency of $Es\text{-}1^{o}$.

Allele notation abbreviated as for Table 5.1.

Mating	Worker genotypes	Worker phenotypes ("nest type")	Expected frequency	Expected frequency of nest type
oo x o	oo	All null	q^3	q^3
+o x o	½+o:½oo	Segregating	$2q^2 - 2q^3$	$2q^2 - 2q^3$
++ x +	++	All +	$(1 - q)^3$	$1 - 2q^2 + q^3$
++ x o	+o	All +	$q(1 - q)^2$	
+o x +	½++:½+o	All +	$2q(1 - q)^2$	
oo x +	+o	All +	$q^2(1 - q)$	

Scanning these to find a sign change locates the point where dL/dq = 0, and two linear interpolations around this point are used to obtain a final value of q.

This process reveals one and only one value of q for each population, as shown in Table 5.3. These results include only the red form of meat ants, since *Es-1*$^{+}$ occurs only rarely or not at all in the other forms studied. Standard errors of q have been derived from the second differential of the likelihood expression, using the relationship

$$\frac{d^2L}{dq^2} = \frac{-1}{\text{Variance of q}} \quad \text{(Mather, 1957)} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

When a value of q is obtained, it can be substituted into the expected frequencies of the three nest types, to test agreement with the observed data. The expected figures given in Table 5.3 show that this agreement is usually very close.

Table 5.3. Gene frequency (q) of *Es-1*^{o} in populations of the red form of meat ants. Expected values in brackets below observed values.

Population	Number of Nests			q ± SE
	Workers all +	Workers segregating	Workers all null	
Dublin	7 (9.26)	9 (4.71)	1 (3.03)	0.56±0.08
Eudunda	10 (10.15)	6 (5.72)	4 (4.13)	0.59±0.07
Arthurton	8 (8.80)	7 (5.57	4 (4.64)	0.63±0.07
Morgan	0 (3.04)	8 (3.70)	8 (9.26)	0.83±0.05
Adelaide	1 (1.11)	2 (1.87)	15 (15.02)	0.94±0.03
Eyre Peninsula	0 (0.34)	1 (0.63)	12 (12.03)	0.97±0.03

5.3. Within and between form variation

The *Es-1* allele frequencies for six populations of the red form of meat ants are shown in Table 5.3, and in the form of a pie-diagram in Figure 5.2. These populations are significantly heterogeneous in the frequencies of the three nest types (χ^2_{10} = 52.3, P<0.001), indicating an underlying heterogeneity in allele frequencies. The populations fall into two groups, with Dublin, Eudunda, and Arthurton in one group, and Morgan, Adelaide, and Eyre Peninsula in the other, with a distinct difference in gene frequencies between the two groups.

Johnson *et al.*, (1969) found that geographic variation in the frequencies of esterase alleles in the harvester ant had a prinicpal component associated with "weather". However, there is no evidence of a simple association between *Es-1*^{o} frequency and climate in the meat ant populations studied here. The "Adelaide" and "Eyre Peninsula" populations are among the most similar with respect to gene frequency, but among the most divergent climatically (*i.e.*, subhumid *vs.* semi-arid).

Table 5.4 shows observed numbers of the three nest types for *Es-1* in forms of meat ants other than red. With three exceptions, the populations appear to be fixed for the null allele *Es-1*^{o}. In two cases, nests of the blue form contain workers of the + phenotype. These workers are in a minority in both cases, two out of 24, and two out of 19. This observation could reflect colony polygyny. Colonies of the blue form often consist of a number of scattered entrance holes, and it is quite possible that such a colony could contain more than one queen. The existence of rare *Es-1*$^{+}$ alleles in this form would then produce segregation ratios similar to those observed.

The figures shown in Table 5.4 for the Morgan population of *viridiaeneus (V)* give a frequency for *Es-1*^{o} of 0.924±0.042, and its nest

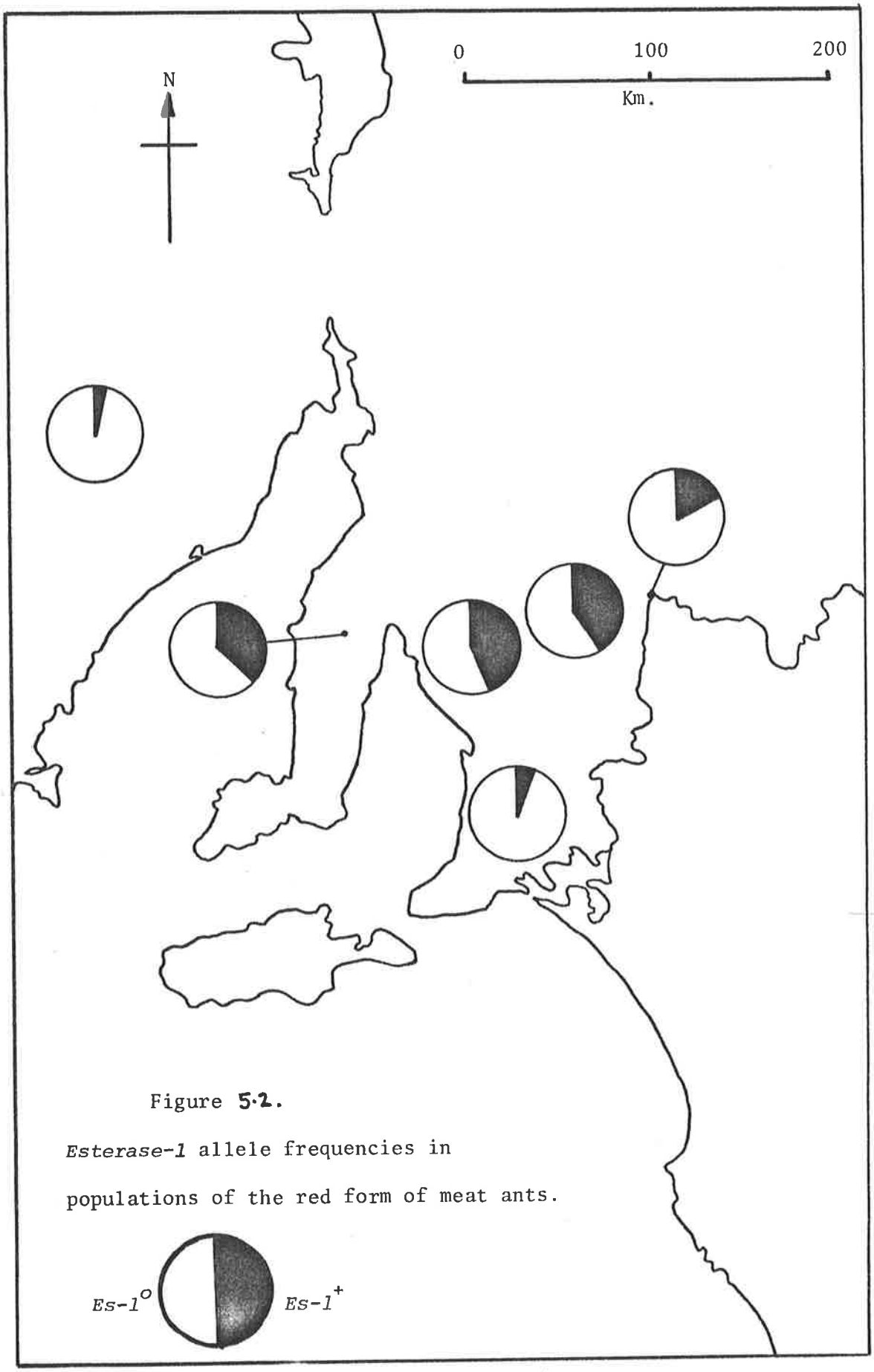


Figure 5.2.

Esterase-1 allele frequencies in populations of the red form of meat ants.

Table 5.4. *Esterase-1* nest types in non-red colour forms.

Place names and abbreviations as in Fig. 3.1.

* see text.

Population	Colour form	Number of nests		
		Workers all +	Workers segregating	Workers all null
Hallett Cove	B	0	0	4
Eyre Peninsula	B	0	0	10
Morgan	B	0	1*	17
Flinders Ranges	B	0	0	7
Snowtown	B	0	0	4
Eudunda	B	0	0	17
Dublin	B	0	1*	16
Eyre Peninsula	V	0	0	5
Morgan	V	2	1	12
Flinders Ranges	V	0	0	8
Eyre Peninsula	SP	0	0	5
Mundoora	SP	0	0	4
Eyre Peninsula	Y	0	0	6

frequencies are not significantly different from those in the sympatric population of the red form. The difference in *Es-1* nest type frequencies between sympatric red and blue form populations is significant at Dublin ($P<0.001$), Eudunda ($P<0.001$), and Morgan ($P<0.01$), which supports the claim that they are reproductively isolated. Other combinations of colour forms, however, cannot be distinguished on the basis of the *Es-1* locus.

5.4. Discussion

The maximum likelihood procedure I have used for the estimation of *Es-1*^{O} allele frequencies depends on a number of conditions. Firstly, it is assumed that the gene frequencies in males and females are the same. This is difficult to test, but a persistent difference in their gene frequencies could only be produced by differential selection. Although male and female ants differ in many ways, it is difficult to see how differential selection at the loci described here could be strong enough to produce detectable changes in the frequencies of nest types.

Secondly, it is assumed that the population is in Hardy-Weinberg equilibrium. Zouros and Krimbas (1969) also found it necessary to make this assumption when using maximum likelihood estimate null allele frequencies in a fruit fly *Dacus oleae*. Although the genotypes of reproductives in meat ants cannot be examined directly, it is possible to compare the observed and expected frequencies of the three nest types. Table 5.3 shows that the agreement between these is usually close, except for apparent excesses of segregating nests at Dublin and Morgan. However, rather than indicating an excess of heterozygotes over the expected frequency, this is just what would be expected if some nests contained more than one queen.

The third assumption of the method, then, is that each nest contains one singly mated queen. It has been shown previously that some nests may contain two or more queens, and the situation is further complicated by the existence of multi-nest colonies. An unknown level of polygyny introduces some uncertainty into the gene frequencies obtained by the maximum likelihood method. Some of these problems could be resolved by extensive excavation of nests, to allow measurement of the level of polygyny, and direct scoring of the phenotypes of males and females. However, it has not been practicable to do this on a large scale for the

purposes of this study. Some idea of the frequency of polygyny of colonies can be obtained by examining workers, and this is attempted in Chapter 8.

The maximum likelihood method as used here applies specifically to a locus with a null allele and a single active allele. Where the null allele is accompanied by a series of active mobility variants, a two-stage procedure has been used, with the frequency of the null allele estimated first (Crozier, 1977b). These two procedures yield the same result for the frequency of the null allele, but the maximum likelihood method has the advantage of providing a variance estimate as well as the gene frequency.

Evidence supporting the model proposed for the genetic control of *Es-1* phenotypes was obtained by typing males (Table 5.1). However, expectations analogous to those shown in Table 5.2 can be derived, and allele frequencies estimated, for a dominant $Es\text{-}1^{O}$ in the same way as for the recessive. The expected frequencies of the three nest types show close agreement with the observations, whether the null allele is considered to be recessive or dominant. Thus, the procedure described here for gene frequency estimation must be based on prior knowledge of the mode of inheritance of the phentoypes, and this evidence cannot be obtained purely from population data on workers.

The observed geographic variation of *Es-1* allele frequencies in the red form of meat ants has probably been produced, in part, by the effects of geographic subdivision of its range, as suggested for *Amylase*. Further discussion of this point is postponed until Chapter 7.

CHAPTER 6

OTHER ISOZYME LOCI IN MEAT ANTS

Apart from *Amylase* and *Esterase-1*, 13 other loci were identified by electrophoresis of meat ant extracts. Most of these loci do not show any variation. They are described below, in alphabetical order.

6.1. *Aldehyde Oxidase (Ao)*

Enzymes with aldehyde oxidase activity have been found to be coded for by several loci in a number of animal species. Genetic variation at these loci has been reported in the spittlebug *Philaeneus spumarius* (Homoptera; Saura *et al.*, 1973), and in *Drosophila* of the *obscura* and *willistoni* groups (Lakovaara and Saura, 1971; Ayala *et al.*, 1974).

Figure 6.1 shows a gel stained for aldehyde oxidase activity after electrophoresis of meat ant extracts (This gel was also stained for *G6pd*; see section 6.5). As with other animal species, each individual shows several zones of aldehyde oxidase activity. In meat ants, no variation has been observed in the pattern shown, in over 600 workers of three colour forms. It is therefore not possible to decide how many loci contribute to this pattern of bands. The most conservative approach is to ascribe it to a single locus, *Aldehyde Oxidase (Ao)*, with a single allele Ao^a.

For this locus, and the others described in the following sections, the number of monomorphic specimens examined per nest was almost always seven or more, so it is unlikely that any segregating nests have been missed.

Figure 6.1.

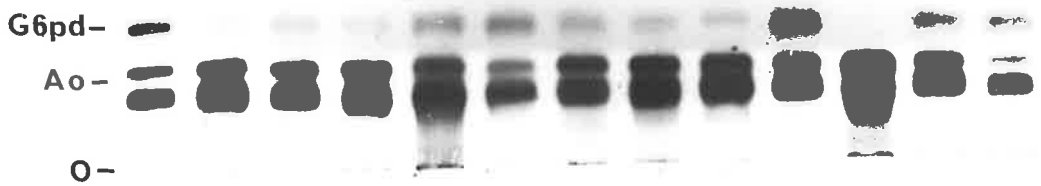
Photograph of a gel stained for aldehyde oxidase and glucose-6-phosphate dehydrogenase. Direction of migration upward.

Slightly larger than actual size.

G6pd = site of *Glucose-6-phosphate dehydrogenase* enzyme activity

Ao = site of *aldehyde oxidase* enzyme activity

O = origin


G6pd–
Ao–
O–

6.2. *Esterase-2 (Es-2)*

In Chapter 5, I discussed genetic variation in the slowest anodal band of esterase activity, and attributed it to the effects of the locus *Esterase-1*. The next slowest band also shows variation of the presence/absence type, which is attributed to the effects of the locus *Es-2* (Figure 6.2 - see also Figures 5.1 and 6.3).

The pattern of segregation shown by males suggested that the presence/absence variation for the *Es-1* enzyme was caused by the existence of a recessive null allele, $Es\text{-}1^{O}$. This evidence is not available for *Es-2*, since males with the null phenotype for this locus have not been found. Nevertheless, the most likely genetic basis for the *Es-2* phenotypes is similar to that for *Es-1* : a recessive null allele $Es\text{-}2^{O}$ and an active allele $Es\text{-}2^{+}$.

An alternative possibility is that the enzymes I have attributed to the loci *Esterase-1* and *Esterase-2*, are actually mobility variants coded for by alleles at a single locus (say "*Esterase-x*"). The allele $Es\text{-}x^{F}$ ("fast") would code for the enzyme I have attributed to *Es-2*, and $Es\text{-}x^{S}$ ("slow") would code for the *Es-1* enzyme. The *Es-1* null phenotype would then be that of a $Es\text{-}x^{F}Es\text{-}x^{F}$ homozygote, when combined with the + phenotype for *Es-2*. This model predicts the existence of $Es\text{-}x^{S}Es\text{-}x^{S}$ homozygotes, which under the two-locus scheme would appear as *Es-1 +, Es-2* null. The expected frequency of nests containing workers of this type can be calculated for each population, since $Es\text{-}x^{F}$ would have the same allele frequency as that derived for $Es\text{-}1^{O}$. The populations of the red form shown in Table 5.3 are expected to include a total of 15.7 such nests, and none were observed. Specimens with the null phenotype for both *Es-1* and *Es-2* could be accommodated by introducing a null allele at the hypothetical locus *Es-x*.

Figure 6.2.

Photograph of a gel stained for esterase activity, showing variation in enzymes coded for by the loci *Esterase-1* and *Esterase-2*.

Direction of migration upward.

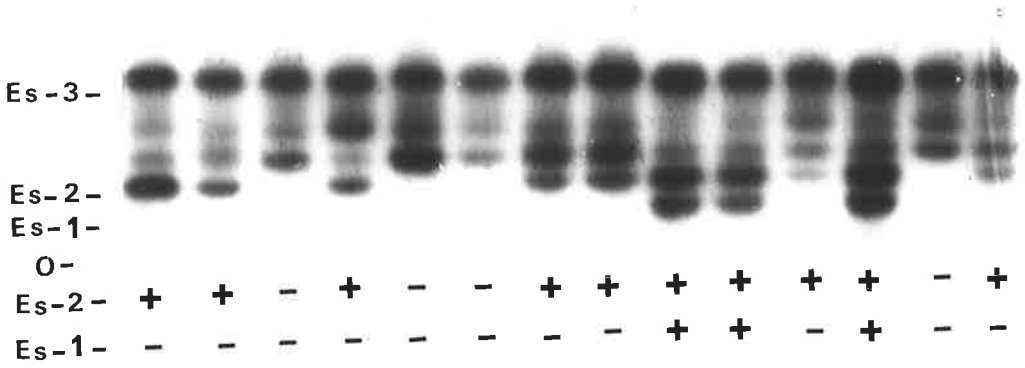
Approximately actual size.

Esterase-1 and *Esterase-2* phenotypes shown as + or -.

Es-3 = site of *Esterase-3* enzyme activity

Es-2 = site of *Esterase-2* enzyme activity

Es-1 = site of *Esterase-1* enzyme activity


Es-3-
Es-2-
Es-1-
O-
Es-2- + + - + - - + + + + + + - +
Es-1- - - - - - - - - + + - + - -

Workers with the *Es-1 +, Es-2* null phenotype would still be expected to appear in this case, but their frequency cannot be simply derived. The fact that no workers of this phenotype were observed out of more than 2000 used for esterase staining argues against the validity of these models.

Specimens with the null phenotype for *Esterase-2* have only been observed in the blue form of meat ants, and only in a total of eight nests, so a detailed analysis is not possible. At Dublin, three nests segregate for *Es-2* out of a total of 17, which gives a gene frequency for $Es\text{-}2^{O}$ of 0.303±0.089, derived in the same way as $Es\text{-}1^{O}$ frequencies. At Morgan, one nest out of 18 contains workers all with the null phenotype for *Es-2*, and two others segregate, giving a gene frequency for $Es\text{-}2^{O}$ of 0.330±0.088. In both these populations the *Es-2* null workers are in a minority in the nests which segregate, (total 86+:21 null), again suggesting polygyny. These gene frequencies for $Es\text{-}2^{O}$ may therefore be over-estimates of its true frequency. The *Es-2* null phenotype also occurs in the small sample of the blue form from Hallett Cove, so $Es\text{-}2^{O}$ may be quite widespread.

Data from colour forms other than blue are not sufficient to rule out the presence of $Es\text{-}2^{O}$. The Dublin red form population contains 17 nests, none of which have any *Es-2* null workers. This gives an upper limit for the true frequency of such nests of 0.198, corresponding to a value of 0.346 for the upper limit of the frequency of $Es\text{-}2^{O}$ (at the 95% level; Stevens, 1942). Thus, gene frequencies at this locus cannot be used as evidence of reproductive isolation between colour forms.

6.3. *Esterase-3 (Es-3)*

The specimens shown in Figures 5.1 and 6.2 all have a single band of activity labelled *Es-3*. Figure 6.3 shows that some specimens have two bands in this region of the gel, and some have a single band with slower mobility. As with *Amylase*, this pattern is characteristic of variation produced by two co-dominant alleles at one locus. The locus is designated *Esterase-3*, with alleles coding for enzymes with fast ($Es\text{-}3^a$) and slow ($Es\text{-}3^b$) mobility. Neither of these enzymes overlaps on gels with the products of other esterase loci, so all three phenotypes can be recognized.

At first, the allele $Es\text{-}3^b$ was only known from two nests of the red form of meat ants, close together at Eudunda. A sample from nest 470 included 9 $Es\text{-}3^aEs\text{-}3^a$ and 11 $Es\text{-}3^aEs\text{-}3^b$ workers, which could be produced by the mating $Es\text{-}3^aEs\text{-}3^b \times Es\text{-}3^a$. Nest 468 contained 22 $Es\text{-}3^aEs\text{-}3^a$:12$Es\text{-}3^aEs\text{-}3^b$:5$Es\text{-}3^bEs\text{-}3^b$, which cannot be explained by one singly mated queen. This nest also shows aberrant segregation ratios for *Es-1* and *Amy*, so polygyny is strongly suggested. It was noted that there were many other nests nearby, so it is possible that nest 468 is part of a multi-nest colony, rather than an isolated nest with more than one queen.

The allele $Es\text{-}3^b$ was subsequently found in another population of the red form, at Belair, 10 km. from Adelaide. This population consists of 20 nests in 16 colonies. Twelve of these colonies contain all (8) $Es\text{-}3^aEs\text{-}3^a$ workers, two contain all $Es\text{-}3^aEs\text{-}3^b$, and two segregate $Es\text{-}3^aEs\text{-}3^a$:$Es\text{-}3^aEs\text{-}3^b$. This gives a gene frequency for $Es\text{-}3^b$ of 0.087, estimated by the maximum likelihood procedure used for *Amylase*. These nests have not been included in the "Adelaide"

Figure 6.3.

Photograph of gels stained for esterase activity.

Direction of migration upward.

O = origin

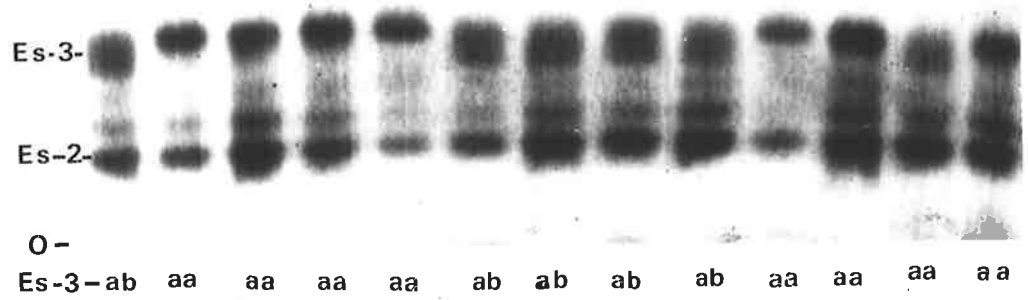
Slightly larger than actual size.

Upper - showing variation in the *Esterase-3* enzyme only. These individuals all have the + phenotype for *Esterase-2* and the null phenotype for *Esterase-1*.

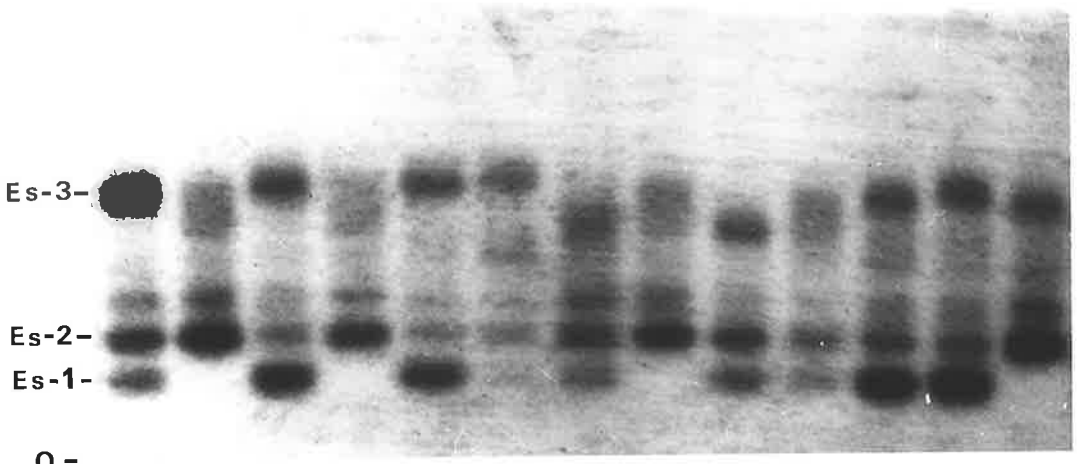
Lower - showing variation in the *Esterase-3* and *Esterase-1* enzymes. These individuals all have the + phenotype for *Esterase-2*. *Esterase-1* phenotypes indicated as + or -

Esterase-3 phenotypes shown in abbreviated notation:

a = $Es\text{-}3^{a}$, b = $Es\text{-}3^{b}$.



O –
Es-3 – ab aa aa aa aa ab ab ab ab aa aa aa aa



O –
Es -3 – aa ab aa ab aa aa bb ab bb ab aa aa aa
Es-1- + – + – + + + – + + + + –

population of the red form referred to earlier, which does not seem to contain *Es-3*^{b}. The significance of these Belair colonies in terms of the social behaviour of meat ants is described in Chapter 8.

The allele *Es-3*^{b} has not been found in any other population surveyed. Its occurrence therefore appears to be highly localized, with considerable differences in frequency between populations which are quite close together. It has also been found only in the red form of meat ants, despite the large number of nests of other forms which have been examined.

6.4. General Protein (*P-1* and *P-2*)

Surveys of isozymes in natural populations often include one or more loci included under the general heading "General Protein", or "Non-specific Protein". They are distinguished from other loci by the fact that their detection does not rely on the enzymatic properties of their products. Detection is by the use of some broad-range protein stain such as Amido Black or Coomassie Blue. This usually reveals several proteins in each specimen, so loci are numbered accordingly.

In the brachiopod *Lyothyrella notorcadensis*, Ayala *et al.* (1975) resolved five such loci, two of which showed genetic variation. The result in the clam *Tridacna maxima* was two loci variable out of four resolved (Ayala *et al.*, 1973), and for 11 larval protein loci, three were polymorphic in *Drosophila miranda*, four in *D. persimilis*, and five in *D. pseudoobscura* (Prakash, 1977).

Staining for general protein in meat ants also reveals multiple zones (Figure 6.4). Coomassie Blue staining revealed more proteins than Amido Black, and there was considerable variation in the intensity of the bands for both stains. Since it is not clear exactly how many proteins can be consistently detected, a conservative score of two was adopted for the number which were clearly resolved. These proteins are labelled P-1 and P-2 on the figure, and they are considered to be coded for by two loci (*P-1*, *P-2*), each with a single allele ($P\text{-}1^a$, $P\text{-}2^a$).

Figure 6.4.

Photograph of a gel stained for general protein.

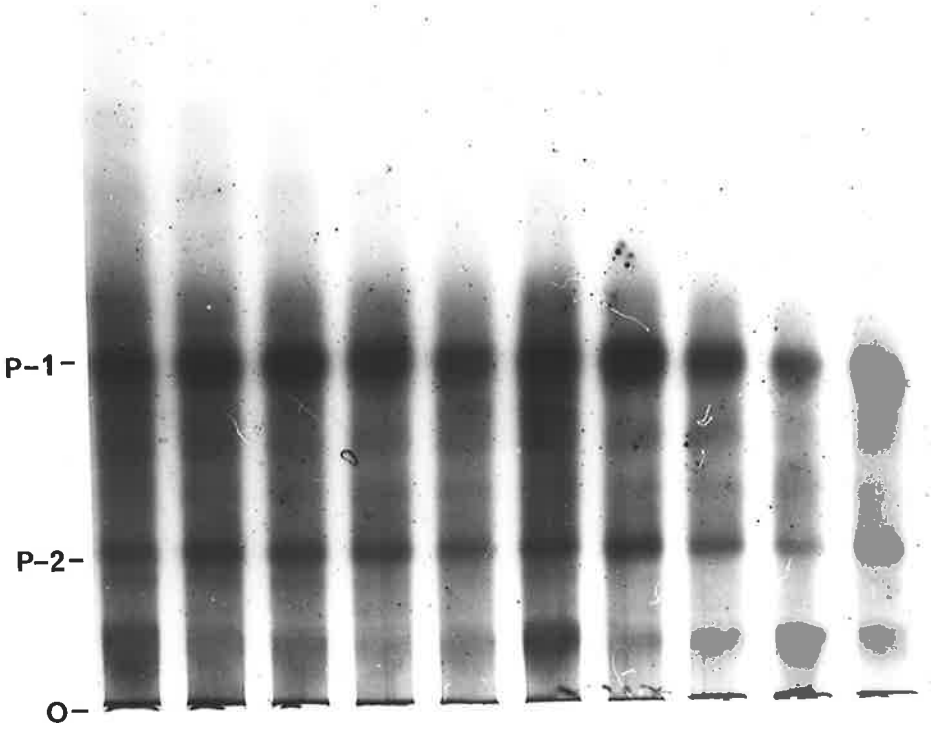
Direction of migration upward.

Slightly larger than actual size.

O = origin

P-1 = "Protein-1" (coded by the locus *P-1*)

P-2 = "Protein-2" (coded by the locus *P-2*)


P–1–
P–2–
O–

6.5. *Glucose-6-phosphate dehydrogenase (G6pd)*

Glucose-6-phosphate dehydrogenase shows genetic polymorphism less frequently than some other enzymes (Powell, 1975). Nevertheless among insects, polymorphism has been reported in cicadas (Krepp and Smith, 1974), and several species of *Drosophila* (*e.g.*, Ayala *et al.*, 1974).

In meat ants, I have examined over 500 workers of three colour forms for this enzyme, and all of them have a single invariant band of activity (Figure 6.1). On some occasions there was apparent variation on poorly resolved gels, but the addition of NADP to the gel and electrolyte buffers improved the resolution, and made it clear that there was no genetic variation present.

This enzyme is then considered to be coded for by a single locus (*G6pd*) with a single allele, $G6pd^a$, in all of the populations examined.

6.6. *Glutamate dehydrogenase (Gdh)*

Glutamate dehydrogenase is an enzyme which is not usually included in isozyme surveys of populations. It has been reported to be monomorphic in the toads *Pelobates syriacus* and *Bufo viridis* (Nevo, 1976), the frogs *Rana ridibunda* (Nevo, 1976) and *Acris crepitans* (Dessauer and Nevo, 1969), and the fish *Zoarces viviparus* (Frydenberg and Simonsen, 1973). Koen and Shaw (1964) found that the lactate dehydrogenase of *Peromyscus* also had activity against glutamic acid, and that a mobility variant in LDH also appeared when stained as GDH.

Both NAD and NADP dependant forms of glutamate dehydrogenase have been reported, but no NADP catalysed activity could be found in meat ants. The results of electrophoresis followed by NAD mediated staining is shown in Figure 6.5. The upper gel shown is an example of a gel which is not well resolved, and which appears to show individual variation. Once again, the resolution of this system is considerably improved by the addition of NAD to the sample extraction fluid, and to the gel and electrolyte buffers. When this is done, the apparent mobility variation is not seen, and each individual has a single zone of activity (lower gel is Figure 6.5). Treatment with NAD does not remove the quantitative variation shown in the intensity of staining, however. A minority of specimens showed extremely weak activity or none at all, and there was a continuous range up to quite strong activity. Only specimens which showed clearly visible staining were scored, and this meant that an increased number of workers had to be run from some nests.

The single band of activity observed is attributed to the locus *Gdh*, with a single allele Gdh^a in more than 500 workers which were scored.

Figure 6.5.

Photograph of gels stained for glutamate dehydrogenase activity.

Direction of migration upward.

Slightly larger than actual size.

Upper - poorly resolved gel containing no NAD in gel buffer.

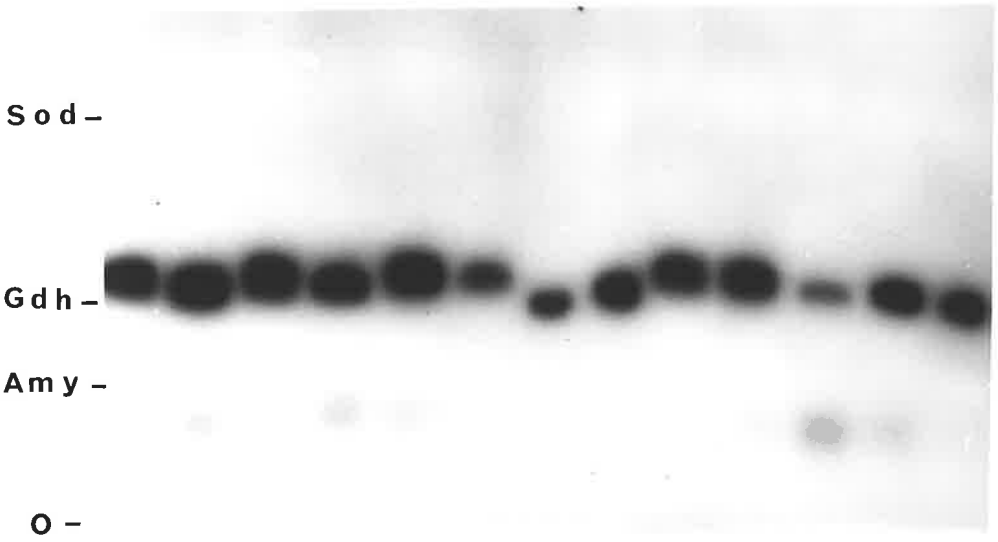
Lower - improved resolution produced by the addition of NAD, indicating that glutamate dehydrogenase has the same mobility in all these specimens.

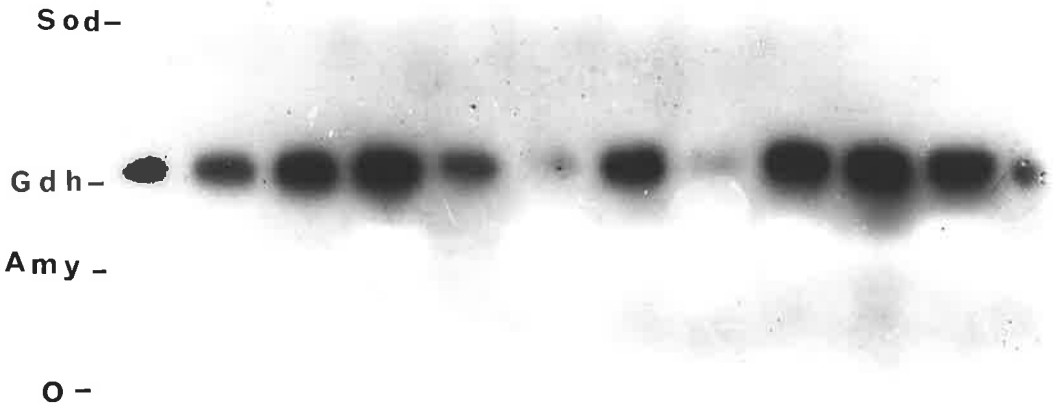
Sod = site of *Superoxide dismutase* enzyme activity

Gdh = site of *Glutamate dehydrogenase* enzyme activity

Amy = site of *Amylase* enzyme activity

O = origin.


Sod –
Gdh –
Amy –
O –


Sod–
Gdh–
Amy –
O –

6.7. *Lactate dehydrogenase (Ldh)*

Lactate dehydrogenase usually appears as multiple zones after electrophoresis. This results from the combined effects of multiple loci, and a quarternary structure which is often tetrameric. Markert *et al.*, (1975) provide an extensive review of the genetic variation in this enzyme in vertebrates, and trace the stages in its evolution. It also shows complex phenotypes in an invertebrate, the horseshoe crab *Limulus polyphemus* (Selander *et al.*, 1970). However, attempts to demonstrate the existence of this enzyme in other invertebrates have not always been successful, and little is known of its occurrence or mode of inheritance in these animals (Manwell and Baker, 1970; Markert *et al.*, 1975).

In meat ants, lactate dehydrogenase appears as a single fairly sharply resolved band of activity in all of the workers tested (over 600). Figure 6.6 shows a gel stained for both lactate and malate dehydrogenases (see Section 6.9). The single lactate dehydrogenase band is taken to indicate the presence of a single allele Ldh^a at the locus *Ldh*, in all the meat ant populations sampled.

Figure 6.6.

Photograph of a gel stained for lactate dehydrogenase and malate dehydrogenase.

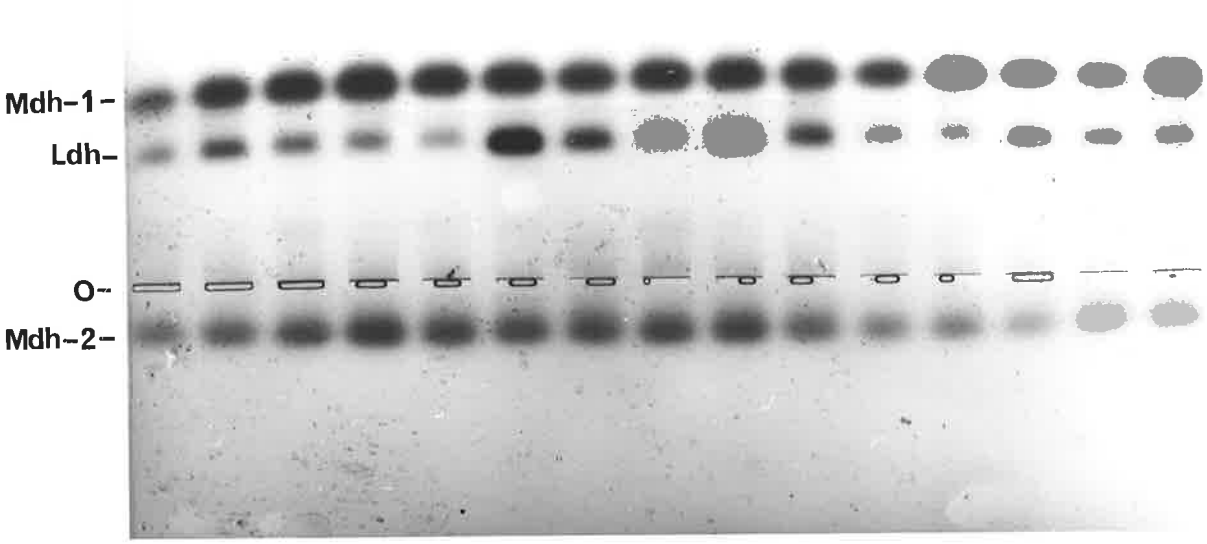
Approximately actual size.

O = origin.

Ldh = site of *Lactate dehydrogenase* enzyme activity

Mdh-1 = site of *Malate dehydrogenase-1* enzyme activity

Mdh-2 = site of *Malate dehydrogenase-2* enzyme activity.


Mdh-1-
Ldh-
O-
Mdh-2-

6.8. *Leucine aminopeptidase (Lap)*

In most organisms surveyed, there are several to many loci which produce enzymes with peptidase activity. The most commonly used substrate is l-leucyl-β-naphthyl amide, so the loci are corespondingly referred to as *Leucine aminopeptidases*. These loci often show genetic polymorphism - *e.g.*, *Lap-5* in several *Drosophila* species (Ayala *et al.*, 1974), a single *LAP* locus in a sawfly (Gorske and Sell, 1976), and two loci in the weevil *Otiorrhynchus scaber* (Suomalainen and Saura, 1973).

Figure 6.7 shows a gel stained for leucine aminopeptidase activity after electrophoresis of meat ant extracts. There is a single main zone of activity in all specimens, which does not show any variation in over 500 workers. This is interpreted as the product of single allele Lap^a at one locus *(Lap)* in all meat ants tested. There is also some staining reaction quite close to the slots, but it could not be resolved clearly after running on several buffer systems, so it is not considered further.

The staining procedure used for peptidase produces orange to red zones of activity, against a yellow background. On some gels, clear zones could be seen where the yellow dye had been de-colourized (Figure 6.7). These may be similar to the white zones found on esterase gels in the blue grouse *Dendrogapus obscurus* and the deer mouse *Peromyscus maniculatus*, which are attributed to the locus *Ng* (Birdsall *et al.*, 1970). They are also similar to the white bands produced by superoxide dismutase on gels stained for dehydrogenase enzymes (see section 6.11). However, since they did not always appear, and are not clearly resolved, I can do no more at the moment than to note the existence of this phenomenon, which may be the result of another enzyme locus.

Figure 6.7.

Photograph of a gel stained for leucine aminopeptidase activity.

Direction of migration upward.

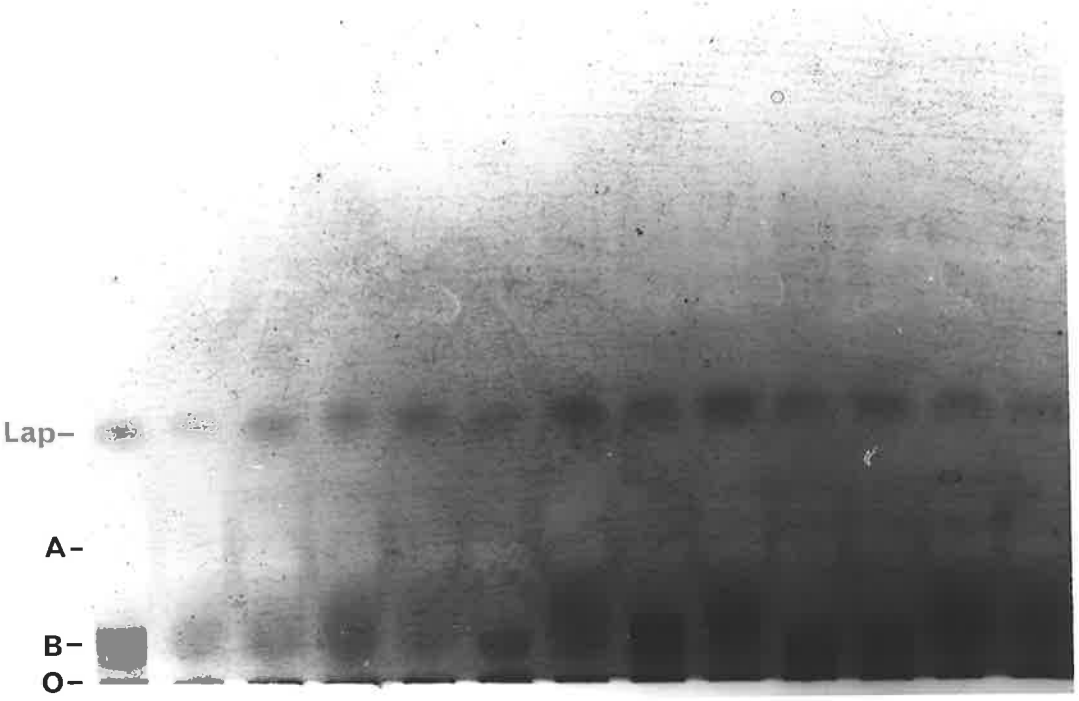
Approximately actual size.

O = origin

Lap = site of *Leucine aminopeptidase* enzyme activity

A = decolourized zones

B = additional poorly resolved leucine aminopeptidase activity near origin.


Lap–
A–
B–
O–

6.9. *Malate dehydrogenase (Mdh-1 and Mdh-2)*

Malate dehydrogenase (NAD dependent) exists in both mitochondrial and supernatant forms in many organisms, and these appear to be produced by different loci (Manwell and Baker, 1970). Best resolution on gels has sometimes been obtained by using a buffer system which causes the two enzymes to migrate in opposite directions. Hence, it is common for them to be referred to as "cathodal" and "anodal" malate dehydrogenase.

The cathodal Mdh (*Mdh-a*) in the ant *Aphaenogaster rudis* shows genetic polymorphism, and also shows three bands of activity in heterozygotes, apparently resulting from the dimeric structure of the enzyme (Crozier, 1973a). In another ant *Pogonomyrmex barbatus*, it is the more anodally migrating enzyme which is variable (Johnson *et al.*, 1969), but in this case, both enzymes run anodally, and the slower one is poorly resolved. Gorske and Sell (1976) report a single polymorphic *Mdh* locus in a sawfly, and *Mdh-2* is polymorphic in several *Drosophila* species (Ayala *et al.*, 1974).

Using a pH 7.0 gel buffer system, both cathodal and anodal malate dehydrogenases in meat ants were sharply resolved (Figure 6.6), and neither enzyme showed any genetic variation in over 600 workers tested. In some cases, specimens which were not handled with sufficient care did show extra bands of activity. These appeared as two or three bands on the anodal side of the main band, decreasing in intensity towards the anode. These are interpreted as "satellite zones" produced by *in vitro* modification of the original enzyme, and not as genetic variants. The phenotypes are therefore attributed to the existence of two loci (*Mdh-1, Mdh-2*), each with a single allele ($Mdh\text{-}1^a$, $Mdh\text{-}2^a$) in all the meat ants tested.

6.10. *Malic enzyme (Me)*

The NADP dependant form of malate dehydrogenase is now commonly referred to as malic enzyme. It is apparently quite distinct from the NAD dependant enzyme, as neither seems to function in the presence of the "wrong" co-factor. Once again there are often at least two loci coding for malic enzyme, which in the mouse, correspond to mitochondrial and cytoplasmic sites of activity (*e.g.*, Selander and Yang, 1969).

The supernatant form of malic enzyme shows genetic polymorphism in *Mus musculus musculus* in Europe, Hawaii and North America (Wheeler and Selander, 1972; Hunt and Selander, 1973), while the mitochondrial enzyme appears to be monomorphic. Ayala *et al.*, (1974) refer to two polymorphic *Me* loci in some species of *Drosophila*, while in others only one locus is reported (Lakovaara and Saura, 1971). There also appears to be a single locus with two alleles in two species of *Aricia* (Lepidoptera) (Jelnes, 1975), and in the spittlebug *Philaenus spumarius* (Saura *et al.*, 1973).

In meat ants, every worker tested had a single band of activity. However, this band did show mobility variation, which is ascribed to the locus *Me*. There are two phenotypes, which are considered to be those of the homozygous genotypes $Me^{a}Me^{a}$ ("fast") and $Me^{b}Me^{b}$ ("slow"), as shown in Figure 6.8. The complete lack of a double or triple banded heterozygous phenotype is surprising, until the geographic and taxonomic distribution of the alleles is considered (Table 6.1). No nests contained workers which segregate for *Me*. Therefore the nests are classified as "all $Me^{a}Me^{a}$" or "all $Me^{b}Me^{b}$" based on at least seven (usually more) workers from each nest. Populations of the red form from Morgan, Arthurton, and Eyre Peninsula all contained only $Me^{a}Me^{a}$ workers, as did Morgan and Eyre Peninsula populations of the

Figure 6.8.

Photograph of gels stained for malic enzyme.

Direction of migration upward.

Approximately actual size.

Me = site of *Malic enzyme* activity.

O = origin.

Upper = Individuals all "Fast" ($Me^{a}Me^{a}$)

Lower - F = "Fast" ($Me^{a}Me^{a}$)

S = "Slow" ($Me^{b}Me^{b}$)


Me –
O –

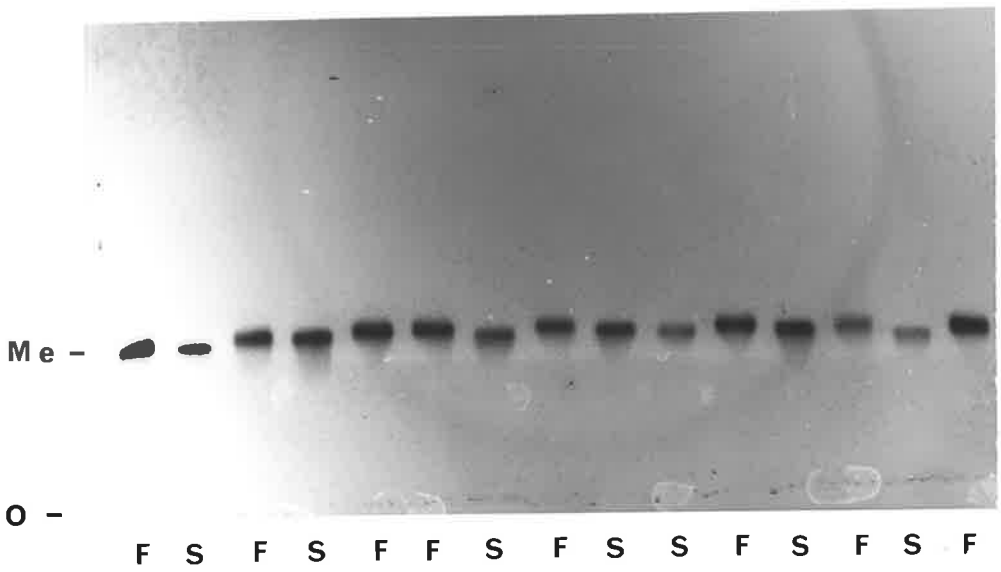

Me –
O –
F S F S F F S F S S F S F S F

Table 6.1. Malic enzyme phenotypes in workers from nests in nine meat ant populations.

Location	Colour form	Number of nests	
		All $Me^{a}Me^{a}$ "Fast"	All $Me^{b}Me^{b}$ "Slow"
Arthurton	P	19	0
Morgan	P	16	0
Morgan	B	17	0
Morgan	V	15	0
Eyre Penin.	P	4	0
Eyre Penin.	B	4	0
Eyre Penin.	V	0	5
Eyre Penin.	Y	4	0
Eyre Penin.	SP	1	3

blue form, and the Eyre Peninsula population of the yellow form. The black form (*viridiaeneus*) population from Morgan is all $Me^{a}Me^{a}$, while that from Eyre Peninsula is all $Me^{b}Me^{b}$. The collection of the small purple form from Eyre Peninsula contained three nests with all $Me^{b}Me^{b}$ workers, and one with all $Me^{a}Me^{a}$ workers.

The specimens in the upper gel in Figure 6.8 are all $Me^{a}Me^{a}$, and include Arthurton red form, Morgan red form and Morgan *viridiaeneus*. Those in the lower gel include all five colour forms from Eyre Peninsula, with $Me^{a}Me^{a}$ individuals of the yellow, red and blue forms, and $Me^{b}Me^{b}$ individuals from the small purple and black (*viridiaeneus*) forms. These gels, like all others, were scored independently several times, without knowledge of the identity of the specimens involved, and always gave the same results.

An interesting confirmation of these results comes from nest 446, an Eyre Peninsula nest of the black form. When sampled, this nest was surrounded by a blue form population, and comprised several relatively widely spaced holes with little or no mound. The workers are fairly small, and in the field their identity was not clear. This nest was found to contain $Me^{b}Me^{b}$ workers, while nearby nests of the blue form contained $Me^{a}Me^{a}$. Close re-examination of preserved specimens later confirmed their classification as black.

The single nest of the small purple form containing $Me^{a}Me^{a}$ workers cannot be accounted for in this way, since close examination of both the nest and the specimens confirm its identity. Thus, this is the only population out of the nine studied in which this locus shows polymorphism, and the lack of heterozygotes and segregating nests may be a chance phenomenon due to the small sample size. The gene frequency of Me^{b} in this population is taken to be 0.75.

Information provided by this locus is of two kinds. The loci considered previously (*Amylase* and the esterases) show geographic variation in allele frequencies. For *Me*, this takes the form of fixation of different alleles in the two populations of the black form considered. Both of these populations are on the extreme edge of the distribution of this form, so they may not be typical of its general composition. Population subdivision on a small scale could also be responsible for the results in the small purple form, since the four nests used are from widely scattered areas.

Secondly, the distribution of alleles among colour forms on Eyre Peninsula gives further evidence of reproductive isolation between them. It is sometimes difficult to distinguish between specimens of the yellow and black forms on morphological grounds, and they cannot be discriminated on the basis of their *Amylase* or *Esterase-1* allele frequencies. However, the fact that they have different alleles at the *Me* locus provides evidence that they are reproductively isolated from each other. The red and small purple forms also differ in their *Me* allele frequencies, although this distinction is less clear.

The existence of both geographic variation within forms, and reproductive isolation between them, indicated by allele frequencies at other loci, are therefore both confirmed by the data obtained from the *Me* locus.

6.11. *Superoxide dismutase (Sod)*

The enzyme now known as superoxide dismutase has been referred to variously as "Tetrazolium oxidase", "Indophenol oxidase", and a number of other names. Its activity appears on many gels stained for dehydrogenases, when conditions are not carefully controlled. Brewer (1967) first described genetic variation for this enzyme in humans, and described the "achromatic zones" produced on the blue background of gels. The formation of this background during staining can be enhanced by bright illumination, use of an alkaline (pH = 8.6) stain buffer, staining at 37°C, and by a high concentration of MTT in the stain. Alternatively, when staining for a dehydrogenase enzyme and the background is undesirable, its formation is reduced by staining in the dark, at pH = 7.5, at room temperature, and with the minimum concentration of stain.

Apart from humans, genetic variation for this enzyme (under various names) has been described in *Drosophila* (Ayala *et al.*, 1974), *Euphydryas* butterflies (McKechnie *et al.*, 1975), and several species of weevils (Suomalainen and Saura, 1973).

Figure 6.9 shows the appearance of a gel stained for superoxide dismutase in meat ants. There is one major zone of activity, which did not vary in more than 600 workers. Other weaker zones sometimes appeared more anodal from the main zone, but they could not be consistently detected. Therefore, the phenotype is attributed to a single locus (*Sod*) with a single allele (Sod^a) in all the meat ants tested.

Figure 6.9.

Photograph of a gel stained for superoxide dismutase.
The specimens shown are all homozygous for the allele *Sod*^{a}.

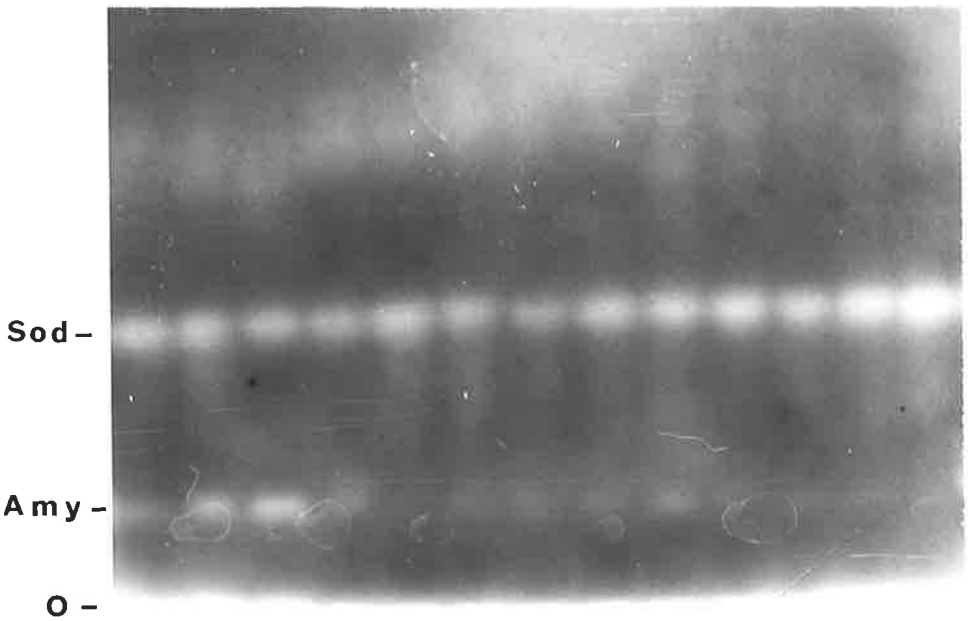
Sod = site of *Superoxide dismutase* activity.

Amy = site of *Amylase* activity.

O = origin.

Direction of migration upward.

Slightly smaller than actual size.


Sod –
Amy –
O –

6.12. *Et alia*

In the search for isozyme loci to use as genetic markers, and in a survey of the level of heterozygosity found in populations, many enzyme stains were tried. This and previous chapters describe the successful systems, - the unsuccessful ones are listed below in three groups.

a) Enzymes which were stained several times, on different buffer systems, for which no activity could be detected:-
alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, peroxidase, and xanthine dehydrogenase.

b) Enzymes which showed some activity in some specimens, but which were not consistently stainable:-
acid phosphatase, aldolase, alkaline phosphatase, fumarase, glutamate oxaloacetate transaminase, hexokinase, and isocitrate dehydrogenase.

c) Enzymes which showed reasonably strong activity in all or most specimens, but which could not be sharply resolved after attempts on several buffer systems:-
α-glycerophosphate dehydrogenase, phosphoglucomutase, and 6-phosphogluconate dehydrogenase.

Given sufficient time and funds, several of these systems could doubtless be developed to the stage where sharp resolution was obtained, and identification of loci was possible. This is especially true for the enzymes in group c, but this undertaking was beyond the scope of the present study.

6.13. Discussion

In this study, as in many other surveys of natural populations, the names given to loci are pragmatic ones, based on the reactions to specific stains. Furthermore, the actual *in vivo* roles of the enzymes are often known only by conjecture, if at all. Nevertheless, for the practical application of enzyme loci as genetic markers, their function is not of the utmost importance. It is important though, that a single enzyme is not counted twice as a result of activity on two or more of the substrates used in stains. This may be of particular significance for dehydrogenase enzymes, some of which are known to act on several substrates (Koen and Shaw, 1964). Often these enzymes can be clearly separated by the fact that variation in one is not reflected in another. In the present case, many of the enzymes do not vary, so this is not possible. Therefore, I have attempted to show by double-staining, that I have not been counting a single enzyme locus twice. This has involved most of the loci used.

a) Aldehyde oxidase.

This enzyme could be detected on gels stained for almost any of the dehydrogenase enzymes, simply by adding a small amount of benzaldehyde to the stain. This quickly resulted in the appearance of extra zones, and the distinctive phenotype of aldehyde oxidase makes it very unlikely that it is being confused with another system.

b) Malate dehydrogenase.

These enzymes are not the same as malic enzyme, since all three systems appeared when both NAD and NADP were added to the stain. Malate dehydrogenase could also be stained on the same slice of gel as lactate dehydrogenase, with the same result. Also, the two banded phenotype of malate dehydrogenase is sufficient to distinguish it from the enzymes coded by *Gdh* and *G6pd*.

c) Malic enzyme

This enzyme could be stained on the same slice of gel as G6pd, Mdh, Ldh and Gdh, and it did not appear in the same position as any of them. Also, the variation observed for malic enzyme did not appear in other enzymes.

d) Glutamate dehydrogenase

Applying a stain containing the substrate for both glutamate and lactate dehydrogenases to a gel resulted in the appearance of both bands, indicating that these enzymes are coded by separate loci.

Some of these double staining systems gave sufficient resolution for the loci concerned to be routinely scored on the same slice of gel *e.g.*, *Mdh-1* and *Mdh-2* with *Ldh*, and *Ao* with *G6pd*. Others did not resolve both enzymes clearly, since they did not involve the optimal gel buffer for both. This in itself gives further evidence that the loci used are indeed all different.

The total number of loci identified is then 15. Five of these have shown some variation in one or more populations - *Amy*, *Es-1*, *Es-2*, *Es-3*, and *Me*, and the patterns of variation in these have been described in this and previous Chapters. The next Chapter examines some aspects of enzyme polymorphism in other animal species, and compares them with the results observed in meat ants.

CHAPTER 7

DISCUSSION - ENZYME POLYMORPHISM IN ANIMAL POPULATIONS

7.1. Patterns of geographic variation.

Isozyme surveys of natural populations often reveal geographic variation in allele frequencies, and the patterns of variation found have been grouped into several classes. Although the classes intergrade to some extent they provide a useful classification of the results, and suggest some of the forces which may be responsible for the observed patterns (Lewontin and Hubby, 1966; Prakash *et al.*, 1969).

a) Complete, or almost complete, momomorphism

Animal populations usually have genetic polymorphism at approximately one third of the loci surveyed (reviewed by Selander, 1976). Loci which are apparently monomorphic over the whole range of a species are therefore very common. In meat ants, the loci *Gdh*, *Sod*, *P-1*, *P-2*, *G6pd*, *Ao*, *Ldh*, *Mdh-1*, *Mdh-2*, and *Lap* appear to be of this type in all the colour forms studied. Various species of animals have also been found to contain loci which are usually monomorphic, but which have rare variants in one or a few populations. The *Ldh-3* locus in *Peromyscus polionotus* has the allele $Ldh\text{-}3^{b}$ fixed or nearly so in most populations, while $Ldh\text{-}3^{a}$ and $Ldh\text{-}3^{c}$ appear in low frequencies in a minority of populations (Selander *et al.*, 1971). *Drosophila willistoni* populations normally contain only one allele ($\alpha\text{-}Gpdh^{1.00}$) for the $\alpha Gpdh$ locus, but the alleles $\alpha\text{-}Gpdh^{0.94}$ and $\alpha\text{-}Gpdh^{1.06}$ also occur in some populations, reaching a maximum frequency of 0.01 (Ayala, 1972). In meat ants, the locus *Es-1* shows this pattern of variation in the blue form (Table 5.4), as well as *Es-3* in the red form (Section 6.3), and *Amy* in the black and small purple forms (Table 4.4).

b) Widespread uniform polymorphism

Surveys of natural populations of *Drosophila* reveal many poly-

morphic loci with very similar allele frequencies in all populations (Ayala, 1972). It has been suggested that this uniformity indicates the action of balancing selection (Prakash *et al.*, 1969; Bulmer, 1973). However, only a small amount of migration between populations may be needed to produce uniform frequencies for neutral alleles as well (Kimura and Ohta, 1971), so balancing selection is not necessarily the only cause of this uniformity. In meat ants, only the *Amy* locus in the blue form has a widespread polymorphism with fairly uniform frequencies in all populations (Figure 4.3).

c) Clines

Many cases are known where the frequency of a gene shows a progressive geographic change, often with different alleles fixed or in high frequency at opposite ends of the species' range. Such gene frequency clines are sometimes correlated with measurable environmental variables, such as temperature, latitude, or altitude, which may suggest the action of natural selection on the polymorphism. Examples of clines and the environmental factors which appear to be influencing them, come from fish (Koehn and Rasmussen, 1967; Merritt, 1972; Christainsen and Frydenberg, 1974), insects (Pipkin *et al.*, 1973, 1976; Johnson, 1976), and molluscs (Boyer, 1974).

However, the mere existence of a gene frequency cline does not necessarily provide evidence of the action of selection. Maruyama (cited by Kimura and Ohta, 1971) has shown that partially isolated populations may tend to have divergent frequencies for neutral alleles, and that they may be connected by populations with intermediate allele frequencies. The resulting pattern may be very difficult to distinguish from a selectively maintained cline.

The meat ant gene frequencies presented in Chapters 4-6 do not show any obvious clines. However, their existence cannot be ruled out,

because of the relatively small number of populations sampled, and their limited geographic distribution. The small purple form occurs from high rainfall areas of Western Australia, to very arid areas in South Australia (Figure 2.4), and this climatic range is accompanied by variation in nest form (see page 18). This situation might offer the best opportunity to detect gene frequency clines in meat ants, and to relate them to environmental variation, but an extensive survey of this type has not yet been attempted.

d) "Patchy" distribution of allele frequencies

Alleles sometimes occur in substantially different frequencies in different populations, but in an unsystematic way. An allele which is usually rare may reach moderate or high frequency in one or a few populations or different populations may be fixed for different alleles. Striking examples of this type of pattern come from the mountain fly *Chamaemyia herbarum* (Chamaemyiidae). For example, the allele *Acph-5* has a frequency of 0.756 in one population , is completely absent from six populations, and has an average frequency of 0.025 in four others (Sluss *et al.*, 1977).

In the pocket gopher *Geomys bursarius*, five loci show considerable variation in allele frequencies between populations, in a way which suggests the action of random drift in small populations (Penney and Zimmerman, 1976). For this process to operate, a species must consist of a series of small sub-populations, between which there is very little gene flow.

In meat ants, patchy distributions of allele frequencies occur at several loci. In the red form, both *Amylase* and *Esterase-1* show considerable differentiation between populations in an apparently unsystematic way (Figure 4.2, 5.2). In the blue form, the allele $Es\text{-}2^{O}$ reaches frequencies of over 0.3 in two populations, and does not appear to be present in others, although its presence cannot be ruled out. In the black form, the two populations which were sampled appear to be fixed for different

alleles at the *Malic enzyme* locus (Section 6.10).

One factor contributing to these patchy gene frequency distributions could be the subdivision of the range of each colour form into separate populations, between which there is little or no gene flow. For the red form, the Eyre Peninsula population is separated from the rest of its range by Spencer Gulf. The Flinders Ranges population is a northerly projection of its range into areas occupied by the black and blue forms. The Eudunda and Morgan populations of the red form are separated by a distance of 50 km., which is occupied exclusively by the blue form.

Similarly, the Dublin and Eudunda blue form populations are separated by 60 km. in which only the red form occurs, and the Hallett Cove population is 60 km. from the nearest other blue form populations.

For the black form, both the Eyre Peninsula and Morgan population are on the extreme edges of its range, so they can also be regarded as somewhat isolated geographically.

This tendency towards geographic fragmentation is likely to be reinforced by meat ants' reproductive behaviour. Greaves and Hughes (1974) found that the mating flights of the red form only take place under a narrow range of environmental conditions (Section 2.5), which may occur over a quite limited area at any one time. Consequently, interbreeding between geographically distant populations may be restricted.

It is not possible to eliminate differential selection in different populations as a cause of gene frequency divergence. Nevertheless, the combination of geographic and behavioural subdivision of range probably means that stochastic processes have played a major role in gene frequency divergence between populations of meat ants.

7.2. Allozymes as taxonomic characters

Evidence obtained from electrophoresis has become a widely used tool in systematics, especially in distinguishing between closely related species. Sibling species have been recognized in this way in organisms as diverse as sea cucumbers (Manwell and Baker, 1963), mosquitoes (Coluzzi and Bullini, 1971), fruit flies (McKechnie, 1975), oligochaete worms (Christiansen and Jelnes, 1976), and lizards (Harris and Johnston, 1976), as well as many others.

If two species can be distinguished electrophoretically it should also be possible to recognize hybrids between them (*e.g.*, Abramoff *et al.*, 1968; Graf *et al.*, 1977; Hung and Vinson, 1977a). Furthermore, once an electrophoretic difference has been established, it can be used as an aid to the identification of subsequent collections (*e.g.*, Ahmad and Beardmore 1976; Buth, 1977). This approach has been used with considerable success for the discrimination of sibling species of *Drosophila* (Ayala and Powell, 1972; Anderson *et al.*, 1977), and results in this genus form the basis of the first formal taxonomic description involving electrophoretic data (Ayala, 1973). It has also been possible to draw up an identification key based on the results of electrophoresis (Avise, 1974).

However, the results of this type of study need to be interpreted with care. The whole approach to species discrimination by the use of allozymes, assumes that the entities which are being recognized are biological species. If two sympatric forms or varieties of a species have different allozymes coded by a particular locus, then this may be taken to mean there is no gene flow between them, and that they are reproductively isolated. Since they are not interbreeding, the two forms may be regarded as separate biological species. To validate this argument, it is therefore necessary to establish two conditions - a) lack of gene

flow, and b) sympatry.

In order to demonstrate a lack of gene flow between two forms, it is first necessary to establish the genetic basis of the variation which is being employed. This is best done by crossing individuals of known genotype, and analysing the genotypes of the offspring, an experiment which is not always possible. Fortunately, the genetic basis of electrophoretic variation is usually simple, and genotypes can be directly inferred from phenotypes.

In cases where two forms of a species appear to be fixed for different alleles at a locus, the genetic basis for the difference may be difficult to establish. The argument for species status in these cases would be strengthened if occasional heterozygous hybrids could be found, or bred in captivity. If breeding data cannot be obtained, the electrophoretic difference between the forms is really only a morphological difference, and the case for their biological separation is weakened.

The electrophoretic variation shown by several enzymes in meat ants seems to have a simple genetic basis. It involves alleles at the loci *Amylase*, *Esterase-1*, *Esterase-2* and *Malic enzyme* (Chapter 4-6), although in the last case the situation is less clear due to the lack of heterozygotes. The fact that sympatric colour forms often have different alleles, or different allele frequencies, for these loci is therefore strong evidence for their reproductive isolation and status as separate biological species.

Their separation on the basis of these loci is sometimes not complete as some colour forms occasionally contain the "wrong" allele - *e.g.*, Amy^{d} in the red form, and Amy^{c} and $Es\text{-}1^{+}$ in the blue form. These rare alleles may indicate that hybrids are sometimes formed. However, the fact that the colour forms maintain separate gene pools, despite the formation of occassional hybrids, strengthens rather than weakening, the

argument for their reproductive isolation.

Geographic variation in allele frequencies within a single species is sometimes great enough to involve the fixation of different alleles in different populations. Therefore, if a lack of gene flow between two forms is to indicate their status as biological species, then they must be sympatric. It is necessary to decide in each case whether the breeding ranges of the two forms overlap sufficiently to allow their breeding individuals to meet (Cain, 1953).

In meat ants, the forms studied are unquestionably sympatric. At Morgan, the red, blue, and black forms occur in extremely close proximity (Figures 2.5, 3.9) and yet gene flow between them is severely restricted (Section 4.4). This is also true of the red and blue forms at Dublin and Eudunda (Figures 3.5, 3.6). On Eyre Peninsula, populations of the five colour forms found there are more dispersed, but many of the closely sympatric pairs of colour forms still do not appear to inberbreed (Table 4.5).

These few loci therefore give good evidence of reproductive isolation between almost all of the five forms studied. Further information of a more general nature concerning their relationships can be obtained from a survey of a larger number of loci. The results of such a survey are presented in Chapter 10.

CHAPTER 8

GENE MARKERS AND SOCIAL BEHAVIOUR

The first step in any survey of genetic variation is the detection of differences among individuals. This Chapter examines some aspects of variation at the individual level in meat ants. In particular the polymorphisms at the *Amylase*, *Esterase-1* and *Esterase-3* loci are used for the analysis of several facets of social behaviour, and for investigating the relationships among the individual members of a nest or colony.

8.1. The origin of males in Hymenoptera

Hymenoptera females have ½ of their genes in common with their sons, but only ¼ in common with their brothers (Crozier, 1970b; Wilson, 1971). Therefore, Hamilton's (1964a, 1964b) genetical theory of the evolution of social behaviour predicts that workers should attempt to lay male-producing eggs themselves rather than allowing their mother to do so. Females (whether workers or queens) should compete for the opportunity to produce males, and this competition may take the form of pheromonal control, eating of eggs, or physical aggression (Wilson, 1971; Hamilton, 1972; Trivers and Hare, 1976).

Furthermore, for a queen to produce males it is necessary that she has some control, during the act of oviposition, over whether a particular egg is fertilized or not. Voluntary control of fertilization seems to be possible in at least one species of Hymenoptera (Gerber and Klostermeyer, 1970), but little information exists concerning the frequency of this phenomenon.

The workers of many species of Hymenoptera lay unfertilized eggs which develop into males, but in other species, males are known to be produced by the queen (reviewed by Wilson, 1971). Evidence concerning the origin of males has come from direct observation of behaviour in the

nest or hive, and the dissection of worker ovaries. It has recently been shown that isozyme polymorphisms provide valuable markers for establishing the parentage of organisms as diverse as lobsters (Nelson and Hedgecock, 1977) and bats (McCracken and Bradbury, 1977), so this approach may also have considerable potential in Hymenoptera.

Crozier (1974) pointed out that in a Hymenoptera nest in which the workers are all heterozygous for two alleles at an allozyme locus, males produced by the queen would all have the same genotype. Males produced by workers would be expected to segregate 1:1. Using the locus *Mdh-a*, he showed that males of the ant *Aphaenogaster rudis* usually developed from eggs laid by the queen. In one nest there did not appear to be a queen, and in this case the males were segregating, indicating that they had developed from eggs laid by workers.

In the honeybee *Apis mellifera*, results from several allozyme loci indicate that males are produced from eggs laid by the queen (Mestriner, 1969; Mestriner and Contel, 1972; Martins *et al.*, 1977). In another bee, *Melipona subnitida*, both workers and queens contribute males to the colony, as indicated by the segregation of alleles at the polymorphic locus *Est-3* (Contel and Kerr, 1976).

In Chapters 4 and 5 I presented evidence from *Amylase* and *Esterase-1* which was consistent with the production of male meat ants from queen laid eggs. A total of seven nests contained potentially informative combinations of genotypes, as shown in Table 8.1. Nests 134 and 418 are the most informative, with workers all heterozygous for *Amylase*, and males which only have a single *Amylase* genotype. These males appear to have been produced from the queen, which in the case of nest 418, was apparently heterozygous at *Esterase-1*. Nests 31, 212, 410, and 411 would have provided information about the origin of males if a sufficient number had been collected. In these nests, the queen would be expected to produce males which segregate 1:1, while the segregation

Table 8.1. *Amylase* and *Esterase-1* phenotypes of workers and males in seven meat ant nests.

Abbreviations:- a = Amy^a *etc.*, + = + phenotype for *Es-1*, - = null phenotype for *Es-1*.

Nest	Workers	Males
31	13dd:7bd	3d:2b
134	22ac	6c
212	6cc:4ac	2a:6c
410	7dd:3bd	2d
411	5dd:3bd	1d
234	12cc+:4cd+ 11cc-:1cd-	9c+:5d+ 4c-:2d-
418	18ac+	2c+:6c-

ratio in worker-produced males would be expected to be 3:1. However, the numbers of males found when these nests were excavated are not large enough to distinguish between these two segregation ratios.

Nest 234 contains a segregation ratio among workers of 23 *Amylase* homozygotes:5 heterozygotes, suggesting the presence of more than one queen. The phenotypes of the workers for *Amylase* and *Esterase-1* are most easily accounted for by the combination of two matings:- $Amy^{C}Amy^{d}$ $Es\text{-}1^{+}Es\text{-}1^{O}$ x $Amy^{C}Es\text{-}1^{O}$, and $Amy^{C}Amy^{C}Es\text{-}1^{+}Es\text{-}1^{O}$ x $Amy^{C}Es\text{-}1^{O}$. The observed segregation ratio among males is 9:5:4:2. The queens in the two above matings would produce males in an expected ratio of 2:2:1:1, which gives a χ^2_3 of 3.25 ($0.50>P>0.30$). The workers would produce males segregating 1.25:3.75:0.25:0.75 ($\chi^2_2 = 15.4$, $P<0.001$; with the two smallest classes pooled). There are several weaknesses in this argument - for instance, it is assumed that the number of queens is not more than two, and that all parents contribute equal numbers of progeny. Nevertheless, in this nest, it appears more likely that the males came from eggs laid by the queens, rather than the workers.

To summarize - although only a small amount of evidence exists, those meat ant nests which do provide any information concerning the origin of males, all point towards their development from unfertilized eggs laid by the queen.

8.2. The number of queens in nests of the red form.

Some of the procedures I have used to estimate gene frequencies in meat ant populations (*e.g.*, for *Amylase* and *Esterase-1*), rely on the existence of one queen in each nest. However, results from the *Amylase* locus (Chapter 4), and from excavation of nests (Greaves and Hughes, 1974) suggest that some red form nests may contain more than one queen. Therefore it is of interest to examine the patterns of genotypes found within nests, to determine whether they yield any information concerning the extent of polygyny, at the level of both nest and colony.

8.2.1. Evidence from *Esterase-1* segregation ratios

If there is a null allele at the *Es-1* locus and if there is one singly-mated queen per nest, any nests which segregate are expected to show a 1:1 ratio of +:null workers, produced by the mating $Es\text{-}1^{+}Es\text{-}1^{O}$ x $Es\text{-}1^{O}$. Also, the segregation ratios in a number of nests should follow the binomial distribution. Populations of the red form included a total of 40 segregating nests, which contained 311 workers with the + phenotype, and 312 null. The distribution of segregation ratios in these nests has been examined among ten workers from each, with ten selected at random in nests where more than this number have been typed. This sample contains 195+:205 null workers. However, Table 8.2 shows that the observed and expected distributions of segregation ratios differ significantly (χ^2_6 = 23.73, P<0.001), and that the observed distribution is rather irregular and over-dispersed, as would be expected if some nests contained more than one queen.

Nests which contain a single queen are expected to be of three types ("All +", "All null", and segregating 1:1). The expected frequencies of these were derived in Chapter 5, in terms of q, the gene frequency of $Es\text{-}1^{O}$. If every nest contains <u>two</u> queens, there are nine possible

Table 8.2. Observed and expected distribution of segregation ratios for *Es-1* in 40 families of size ten.

Number of + workers out of 10	Expected number of nests		Observed number of nests		Direction of deviation
10	0.039		0		
9	0.391	2.188	1	4	+
8	1.758		3		
7	4.688		5		+
6	8.203		10		+
5	9.844		4		-
4	8.203		8		-
3	4.688		1		-
2	1.758		5		
1	0.391	2.188	2	8	+
0	0.039		1		

combinations of segregation ratios among their broods, and the frequencies of these are shown in Table 8.3. These nine combinations reduce to five nest phenotypes, and the additional two types are those which show 1:3 and 3:1 segregation ratios. The expected frequencies of these five nest types are shown in Table 8.4. The two queens producing the "First Brood" and "Second Brood" are assumed to have independent genotypes, and to produce workers at equal rates.

The three types of segregating nests are expected to appear in a population in proportions a:b:c which depend on the gene frequency in that population. Table 8.5 shows representative examples of these proportions. In practise, each of the three types of segregating nests will be represented by a binomial distribution when samples are collected. These binomial distributions are shown in Table 8.6. The overall distribution of segregation ratios to be expected in a population can then be obtained by combining Tables 8.5 and 8.6. For example, a ratio of 3+:7 null, in a populations where q = 0.6, is expected to occur with a frequency of

$$(0.003090)(0.285696) + (0.117188)(0.297216) + (0.250282)(0.124416)$$
$$= 0.066852.$$

In this population, the proportion of nests which segregate is 0.707328. Therefore, in a sample of 100 segregating nests from this population, $(0.066852 \times 100)/(0.707328) = 9.45$ are expected to segregate 3+:7 null. On this basis it is possible to derive the expected distribution of segregation ratios in any population, and to determine how it is affected by changes in q.

I have done this for 19 values of q ranging from 0.01 to 0.99, with the aid of computer program QUEEN. Representative distributions are presented as histograms in Figure 8.1, considering 100 segregating nests in each case. When q is low, the distribution is skewed in favour

Table 8.3. Expected frequencies of the nine possible combinations of broods produced by two unrelated queens; and the resulting segregation ratio among the workers in each case in terms of q, the gene frequency of the null allele.

Second brood \ First brood	All null q^3	Segregating 1:1 $2q^2 - 2q^3$	All + $1 - 2q^2 + q^3$
All null q^3	All null q^6	1 + :3 null $q^3(2q^2 - 2q^3)$	1:1 $q^3(1 - 2q^2 + q^3)$
Segregating 1:1 $2q^2 - 2q^3$	1+ : 3 null $q^3(2q^2 - 2q^3)$	1:1 $(2q^2 - 2q^3)^2$	3+ : 1 null $(2q^2 - 2q^3)(1 - 2q^2 + q^3)$
All + $1 - 2q^2 + q^3$	1:1 $q^3(1 - 2q^2 + q^3)$	3+ : 1 null $(2q^2 - 2q^3)(1 - 2q^2 + q^3)$	All + $(1 - 2q^2 + q^3)^2$

Table 8.4. The five possible nest phenotypes produced by equal contributions of two queens per nest, and their frequencies in terms of q, the gene frequency of the null allele.

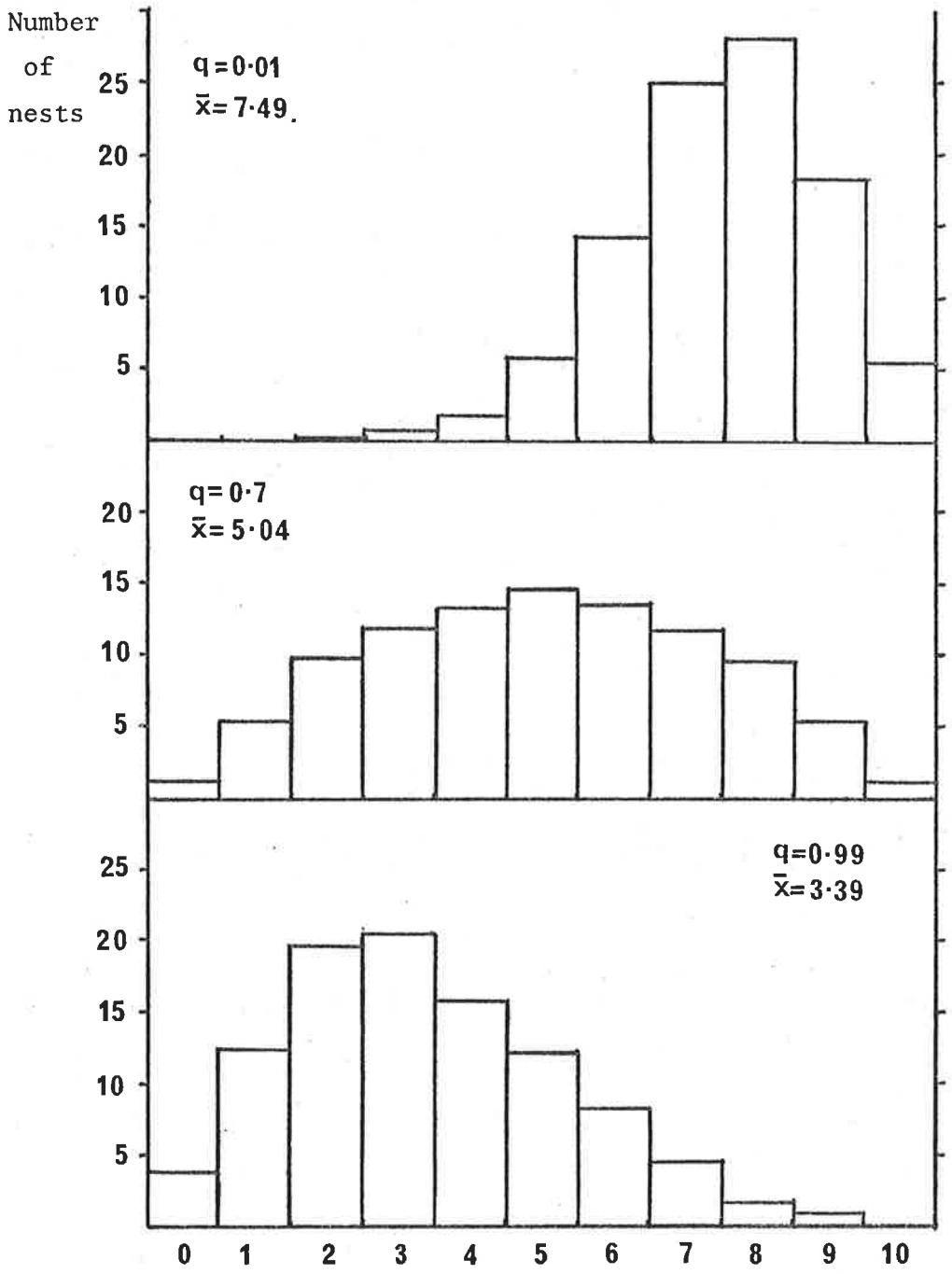
Workers +:null		Expected frequency
1:0		$1 - 4q^2 + 2q^3 + 4q^4 - 4q^5 + q^6$
3:1	a =	$4q^2 - 4q^3 - 8q^4 + 12q^5 - 4q^6$
1:1	b =	$2q^3 + 4q^4 - 12q^5 + 6q^6$
1:3	c =	$4q^5 - 4q^6$
0:1		q^6

Table 8.5. Expected frequencies of the three types of segregating nests produced by two queens in each nest, as a function of the gene frequency of the null allele.

	Segregation ratio +:null			Total
	3:1	1:1	1:3	frequency of
q	a	b	c	segregating
				nests
0.01	0.000396	0.000002	0.000000	0.000398
0.10	0.035316	0.002286	0.000036	0.037638
0.30	0.213444	0.061614	0.006304	0.281862
0.50	0.312500	0.218750	0.062500	0.593750
0.60	0.285696	0.297216	0.124416	0.707328
0.80	0.118784	0.303104	0.262144	0.634032
0.90	0.035316	0.185166	0.236196	0.456678
0.99	0.000396	0.019982	0.038040	0.058418

Table 8.6. Frequency distribution of samples of size ten produced by varying segregation ratios.

Workers in a sample of 10		Segregation ratio +:null		
+	null	3:1	1:1	1:3
0	10	0.000001	0.000977	0.056314
1	9	0.000029	0.009766	0.187712
2	8	0.000386	0.043945	0.281568
3	7	0.003090	0.117188	0.250282
4	6	0.016222	0.205078	0.145998
5	5	0.058399	0.246094	0.058399
6	4	0.145998	0.205078	0.016222
7	3	0.250282	0.117188	0.003090
8	2	0.281568	0.043945	0.000386
9	1	0.187712	0.009766	0.000029
10	0	0.056314	0.000977	0.000001



Number of *Es-1* + workers out of 10 per nest.

Figure 8·1

The distribution of *Esterase-1* segregation ratios in 2-queen nests, as a function of q, the gene frequency of *Es-1*^{O}.Gene frequency and resulting distribution mean indicated in each case, considering 100 segregating nests in each population.

of the + phenotype, and when q is high, it is skewed in favour of the null phenotype, as seems intuitively reasonable. When q is close to 0.7, the distribution is almost symmetrical, and its mean is close to the value of five + individuals out of ten per nest, as expected if each contained one queen. At q = 0,707, the mean is 5.001. The relationship between q and the mean of the resulting distribution is shown in Figure 8.2 for 13 values of q.

Clearly both the skewness and the mean of this distribution depend heavily on the gene frequency. Therefore, if populations with differing gene frequencies are pooled, the overall distribution of segregation ratios need not be symmetrical, and need not have a mean of 1:1, as it would if each nest contained one queen. In populations where only some nests are polygynous, the result is difficult to predict. This is especially true when multi-nest colonies are included in the data. It was shown in Chapter 2 that a single meat ant colony sometimes contains a number of nests which exchange workers freely. If such a colony contained several queens, a sample of workers from one nest would be a complex mixture of genotypes with an unpredictable segregation ratio (see Section 8.4).

Therefore the apparently aberrant distribution of segregation ratios shown in Table 8.2 has probably been produced by the pooling of results from a number of populations which differ in gene frequency, and by the fact that the data included some polygynous nests and multi-nest colonies. The fact that the overall segregation ratio of 195:205 is very close to 1:1 may be fortuitous. A mixture of populations which have an overall mean gene frequency for the null allele of near 0.7 could produce a mean segregation ratio near 1:1, even if every nest contained two queens (Figure 8.2). The populations from which the data on segregation ratios were obtained do indeed have a mean gene frequency

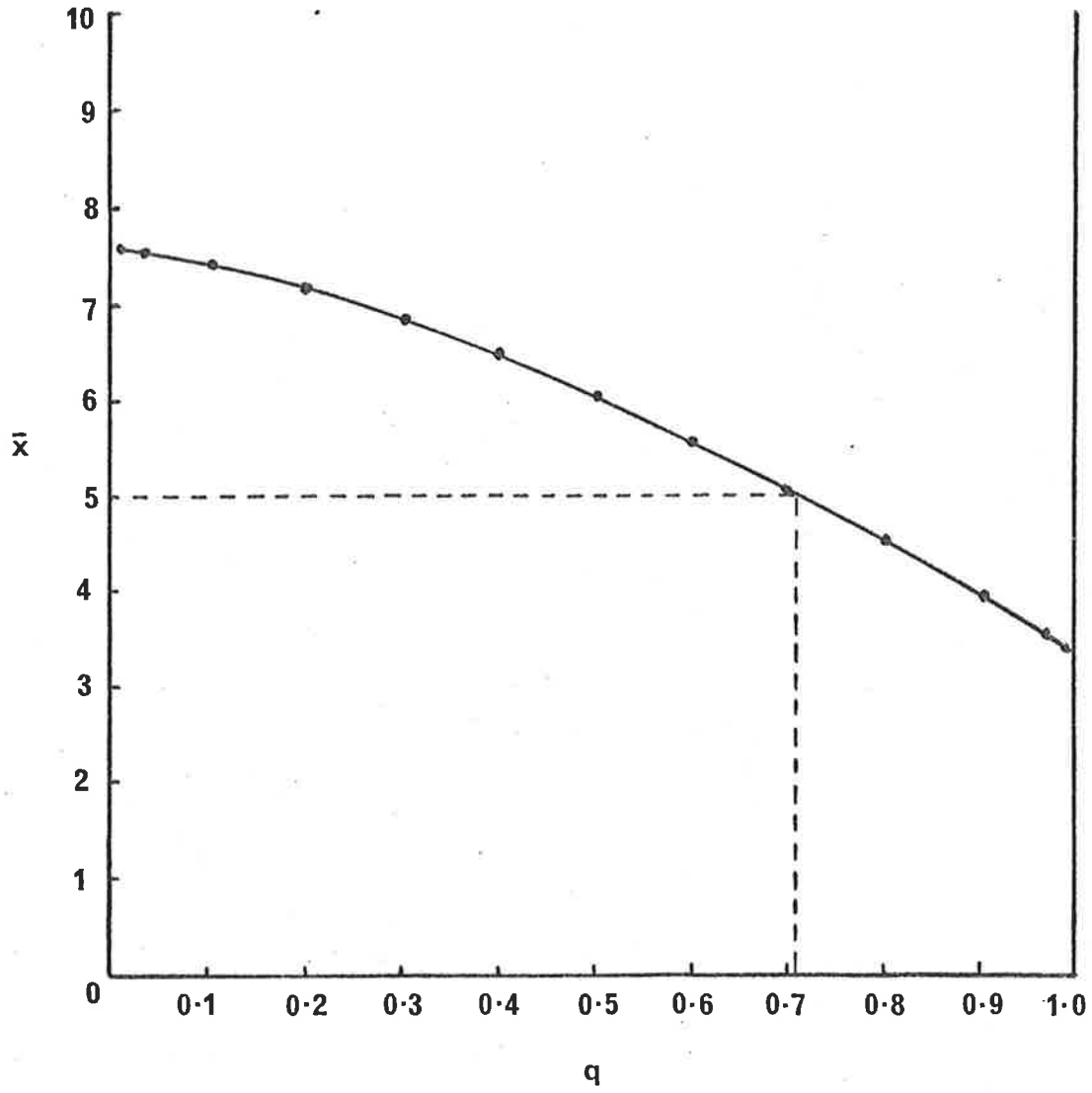


Figure 8·2

The mean of the expected distribution of *Esterase-1* segregation ratios, as a function of q, the allele frequency of *Es-1*^{O}. Every nest is considered to contain 2 queens. $\bar{x}$ = mean of distribution. Dashed line indicates readings when $\bar{x}$ = 5, out of a total of 10 workers per nest.

of about 0.7, as shown in Chapter 5 (Table 5.3).

To analyse this problem further, a large number of segregating nests from a single population should be sampled. However, the maximum number of such nests in a population available at the moment is nine, which is insufficient to allow this analysis. Nevertheless, the data presented here are consistent with the suggestion that in the red form of meat ants, some nests and colonies contain more than one queen.

8.2.2. Evidence from the *Amylase* Locus

A nest founded by a single queen should contain workers with either one or two genotypes for the *Amylase* locus. The nests which contain two genotypes are expected to contain heterozygotes and homozygotes in equal frequencies. It was shown in Chapter 4 that a third *Amylase* genotype sometimes appears in a nest. In the red form, this usually means that a nest contains $Amy^{a}Amy^{a}$, $Amy^{a}Amy^{c}$, and $Amy^{c}Amy^{c}$ workers. Another possibility is that a nest contains the two different homozygous genotypes such as $Amy^{a}Amy^{a}$ and $Amy^{c}Amy^{c}$, and no heterozygotes. Thus, polygyny may manifest itself by the production of recognisable combinations of genotypes in a nest, and not just by changes in their frequencies, as it does for *Esterase-1*.

Table 8.7 shows all possible combinations of genotypes produced by equal contributions of two queens to each nest. Many of the resulting worker groups either do not segregate, or segregate 1:1 in a way which cannot be distinguished from an array of workers produced by a single queen. Many of the two-queen combinations produce 3:1 segregation ratios among the workers. However, with the complicating factors of unequal contributions from the two queens, and random sampling factors, it is not practicable to discriminate between 1:1 and 3:1 ratios.

Table 8.7. Combinations of genotypes of workers produced by two queens per nest, in a population containing the alleles *Amy*^{a} (a) and *Amy*^{c} (c).

\- = combination which can be accounted for by one queen;
* = combination showing a 3:1 segregation ratio;
** = combination which cannot be produced by one singly mated queen.

	Second mating					
First mating	aa x a	aa x c	ac x a	ac x c	cc x a	cc x c
aa x a	-	-	*	**	-	**
aa x c	-	-	*	*	-	-
ac x a	*	*	-	**	*	**
ac x c	**	*	**	-	*	*
cc x a	-	-	*	*	-	-
cc x c	**	-	**	*	-	-

It is possible though, to identify the two-queen combinations which produce arrays of genotypes which cannot be produced by a single queen. These are shown ** in Table 8.7. They all involve the existence of two different homozygous genotypes in the same nest.

If the population is in Hardy-Weinberg equilibrium, then the total frequency of these recognizable two-queen nests is expected to be

$$k = 4q^4(1-q)^2 + 6q^3(1-q)^3 + 4q^2(1-q)^4$$

where q is the frequency of *Amy*^{a}. It is therefore possible to evaluate this quantity for each population surveyed, and to compare the observed and expected numbers of these recognizable nests. The results are shown in Table 8.8.

For the red form, the recognizable nests are expected to be quite rare, due to the low frequency of *Amy*^{a}. A total of five was observed, compared with an expectation of 4.2, and four of these five come from the single Morgan population. This population contains a high proportion of multi-nest colonies of the red form, one of which is examined in detail in Section 8.4. Polygyny in such colonies may involve the existence of queens in different nests throughout the colony, rather than in one nest. Nevertheless, from the results shown in Table 8.8, the possibility that a high proportion of red form nests contain two or more queens, cannot be excluded.

Of course, there is no reason why the number of queens in a nest or colony should be restricted to two. Greaves and Hughes (1974) found one nest with four queens as well as several with two. However, with the allozyme data available at the moment, only two can be detected. It would be of considerable interest to repeat the above analysis in populations containing no multi-nest colonies. However, by the time nests are large enough and mature enough to contain more than one queen

Table 8.8. Frequency of red form nests containing workers of two different homozygous genotypes for the *Amylase* locus.

Exp. = expected number of such nests calculated on the assumption that every nest contains two queens (k x N).

Obs. = observed number.

Population	Number of nests (N)	k	Exp.	Obs.
Barossa Valley	13	0.0091	0.119	0
Eyre Penin.	14	0.0564	0.790	0
Arthurton	19	0.0012	0.024	0
Dublin	17	0.0015	0.026	0
Eudunda	20	0.0370	0.741	0
Morgan	16	0.1163	1.861	4
Flinders Ra.	5	0.0942	0.471	1
Southeast	5	0.0151	0.076	0
Upper Murray	4	0.0223	0.089	0
			4.197	5

most of them will probably have developed into multi-nest colonies as well, so it may not be possible to distinguish between polygyny at the level of the nest and the colony in this way. However, further information about the structure of small and large colonies can be obtained, as presented in the remainder of this Chapter.

8.3. Structure of a group of small colonies of the red form.

The existence of territorial behaviour in ants has been described on many occasions (*e.g.*, Elton, 1932; Brian, 1965; Sherba, 1964; Wilson, 1971). In Chapter 2 I described some aspects of the behaviour of meat ants, and in particular it was shown that they are territorial, and that territorial boundaries can be reocgnized by the characteristic confrontation behaviour shown when ants from different territories meet.

If territoriality is strictly obeyed, it should be possible to show that the workers in neighbouring colonies sometimes have different genotypes, and that sudden changes in genotype frequencies occur across territorial boundaries.

The population chosen for this experiment is situated near Belair, 10 km. from Adelaide, and has been described in detail by Greenslade (1975a, 1975b). The population occupies an area of about 200 x 200 metres, on a small ridge with a flat plateau crest. It is bounded on three sides by dense vegetation in which meat ants do not build nests, and on the fourth side by a railway cutting. It is therefore isolated from contact with nearby populations and provides a good opportunity to examine inter-nest relationships.

In January 1971, the population included 27 colonies, with a total of 62 nests. Of these nests, 33 were permanently occupied, 6 temporarily occupied, and 23 abandoned. By 1973 there were 26 occupied nests, with the reduction caused by the combined effects of human interference and encroaching vegetation (Greenslade, 1975b). The disposition of nests and colonies in 1977 is shown in Figure 8.3. The population then contained 20 nests in 16 colonies, and there had been some re-arrangement of territories and establishment of new nests, as well as the abandonment of old ones, since 1973.

Eight workers from each of the 20 nests were typed for *Amylase*

Figure 8·3

Location of nests and territories in a population of the red form of Meat Ants.

• Nest location.

..... Trails between nests.

— — — Approximate territorial boundaries 22/9/77.

Railway line.

Railway cutting.

$\frac{5}{11}\,\frac{16}{}$ Nest identification.

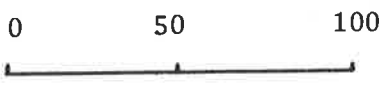
Upper number : numbering in present series.

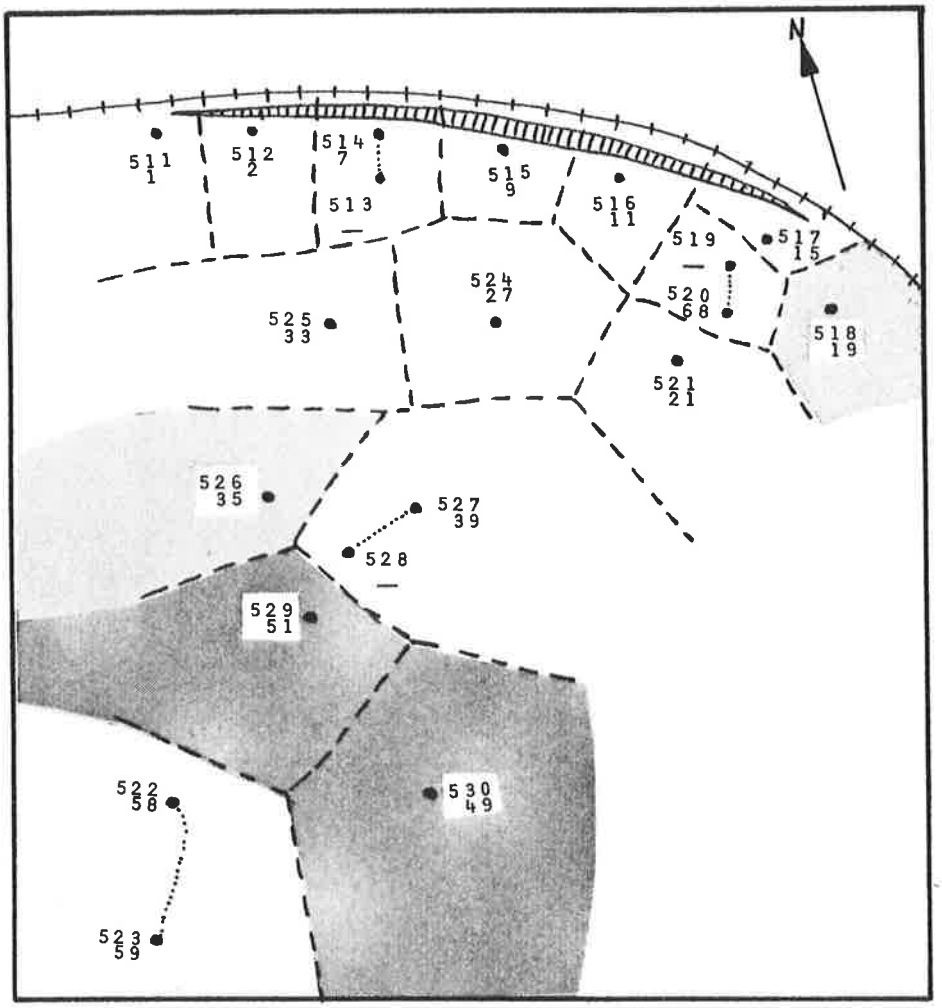
Lower number : numbering as used by Greenslade (1975). Where this is shown as a dash the nest did not exist at that time.

Colonies containing workers which segregate $Es\text{-}3^{a}Es\text{-}3^{a}$:$Es\text{-}3^{a}Es\text{-}3^{b}$

Colonies containing workers which are all $Es\text{-}3^{a}Es\text{-}3^{b}$ heterozygotes

Colonies containing workers which are all $Es\text{-}3^{a}Es\text{-}3^{a}$ are unshaded.




N
511
1
512
2
514
7
513
515
9
516
11
519
517
15
524
27
520
68
518
19
525
33
521
21
526
35
527
39
528
529
51
530
49
522
58
523
59

and the esterases. *Amy*, *Es-1* and *Es-2* did not show any polymorphism, but *Es-3* had two alleles in this population. The genotypes found among these workers are shown in Table 8.9.

Most nests contain only $Es\text{-}3^{a}Es\text{-}3^{a}$ workers, including all of the four pairs of nests from two-nest colonies. However, the existence of $Es\text{-}3^{b}$ in some colonies allows them to be distinguished from neighbouring ones.

Nest 518 contained workers segregating $Es\text{-}3^{a}Es\text{-}3^{a}$: $Es\text{-}3^{a}Es\text{-}3^{b}$, which distinguishes it from the nests in three neighbouring colonies (Figure 8.3). Nest 526 also contains segregating workers, while the nests in two adjacent colonies (525, and 527+ 528) contain only $Es\text{-}3^{a}Es\text{-}3^{a}$ workers. A third colony adjacent to nest 526 contains only $Es\text{-}3^{a}Es\text{-}3^{b}$ workers (nest 529). Nest 530 also contains only heterozygous workers, while those in the neighbouring colonies (nests 522 + 523, and 527 + 528) contain only $Es\text{-}3^{a}Es\text{-}3^{a}$ workers. The trail connecting nests 527 and 528 is a weak one, passing through very dense undergrowth. When examined in the field, it was not possible to decide definitely whether nest 528 was a satellite of 527 or 529. The *Es-3* results indicate clearly that nest 528 is exchanging workers with 527, and not with 529.

In no case do nests connected by a trail contain workers of different genotypes, but in several cases, nests separated by territorial boundaries do contain workers with markedly different genotype frequencies. Thus, the visual impression that these nests are not exchanging workers is completely confirmed.

The Belair population described here has colonies which contain only one or two nests each, but the number of nests in a colony is sometimes much more than this. The next section examines a colony at the other extreme, containing over 80 nests, which appear to exchange workers freely.

Table 8.9. *Esterase-3* genotypes of workers from nests of the red form from Belair. Nests within colonies are bracketed.

Nest number	$Es\text{-}3^{a}Es\text{-}3^{a}$	$Es\text{-}3^{a}Es\text{-}3^{b}$
511	8	0
512	8	0
513	8	0
514	8	0
515	8	0
516	8	0
517	8	0
518	5	3
519	8	0
520	8	0
521	8	0
522	8	0
523	8	0
524	8	0
525	8	0
526	6	2
527	8	0
528	8	0
529	0	8
530	0	8

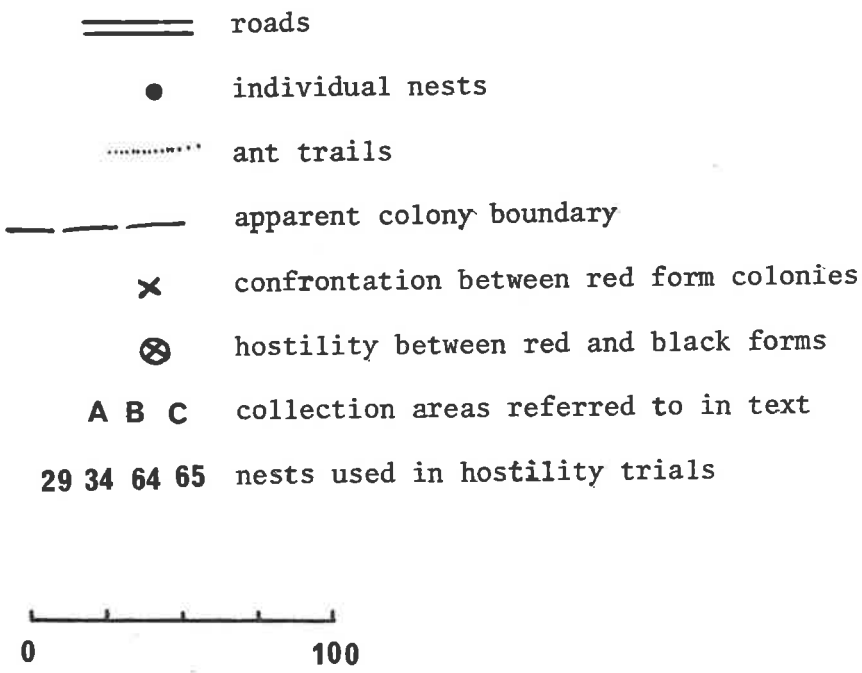
8.4. Structure of a large colony of the red form

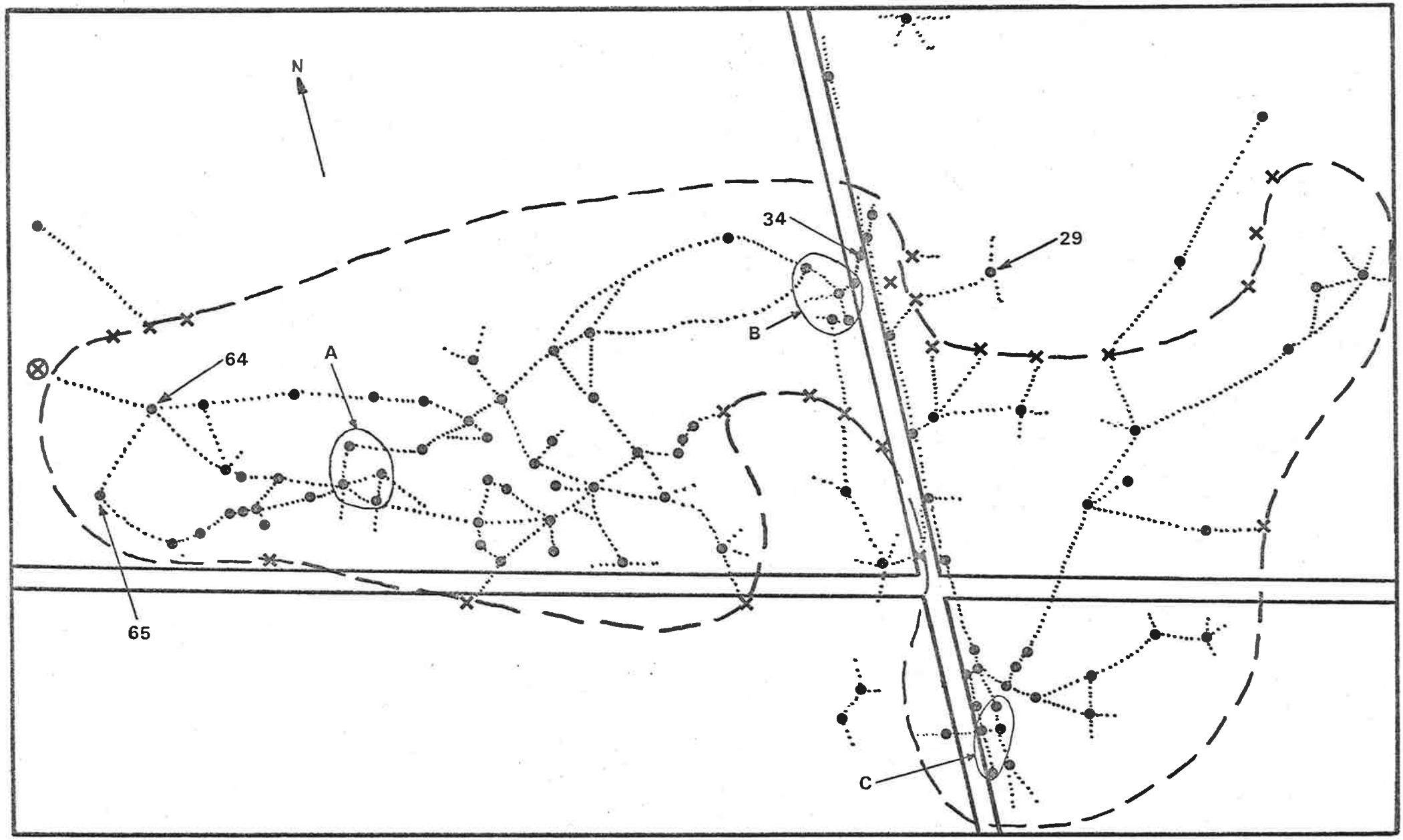
Figure 8.4 is a map of a large colony of the red form, near Morgan, South Australia. This colony is located in the southern part of the area shown in Figure 2.5. It is bounded on the west by an area of open pasture, and by contact with a colony of the black form. Elsewhere, its perimeter is marked by confrontation with neighbouring red form colonies. The colony covers over ten hectares of ground, and includes at least 86 nests. The nests range from one hole to over 200 holes each, with most in the range 20-40 holes. The total number of open entrance holes counted in November, 1976 was 1688, and the colony probably has a population of several million individuals.

The nests are interconnected by a complex system of trails, which often follow the tracks made by sheep. Some of the trails are very conspicuous and carry heavy traffic in both directions, while others are weakly marked and difficult to trace. These trails make it obvious that there is a considerable amount of inter-nest movement by workers during the day, when they are active.

Figure 8.4 includes four nests labelled 29, 34, 64 and 65. These were used to examine the effect of the large size of the colony, on the territorial behaviour of its members. Between three and six workers from each of these four nests were placed near each of the other nests and the reactions of the occupants noted. Workers were also collected, handled, and returned to their own nest. Transferring ants between nests sometimes resulted in hostile interactions, which included several aspects of the usual confrontation behaviour seen between members of different colonies - adoption of a stiff-legged stance, raising of the gaster, and occasionally, physical attacks. Alternatively, the newcomers were accepted after a brief examination and allowed to remain, with no sign of any hostility. A minority of workers were lost, or

Figure 8.4. Map of a colony of the red form of meat ants, near Morgan, South Australia.




N
34
29
B
64
A
65
C

fled the scene, before any reaction (or lack of it) could be observed. The results are shown in Table 8.10.

Workers were always accepted quickly when returned to their own nest, and those from nests 34, 64 and 65 were also acceptable to each other. Ants from nest 29 were not tolerated by those from any other nest, and this hostility was reciprocated. Nests 34 and 29 are only 60 metres apart, but their occupants are hostile to each other both naturally (Figure 8.4), and when artificially brought together. Ants at nests 64 and 65 accepted those from 34, but only after a thorough examination.

These results indicate that the colony enclosed by a dashed line in Figure 8.4 is a single unit as suggested, and that nest 29 is not included in this unit. There also seems to be a tendency towards local subdivision of the colony, such that the workers from widely separated nests may not recognize each other as readily as those from neighbouring nests, but larger-scale experiments are needed to confirm this.

It was suggested in Chapter 2 that the ants in a multi-nest colony mix freely during the day, but return to the same nest at night. If this is so, and if there is a number of queens of varying genotype scattered throughout the colony, then workers collected from a single nest during the day and at night, would be expected to differ in genotype frequencies in some cases. Also, night collections taken from several nests throughout the colony should be more heterogeneous in genotype frequency, than daytime collections from the same nests. The alternative hypothesis is that workers move freely between nests during the day, and spend the night in whichever nest they are near at nightfall. The colony is then a single homogeneous, unstructured unit. The workers from a series of nests in various parts of the colony would then be expected to have similar arrays of genotypes, whether collected during the day or at night. Also, day and night collections from a single nest

Table 8.10. Reactions between workers from four nests of the red form of meat ants.

- = ant examined with antennae and accepted with no hostility;
+ = hostile interaction with raising of gaster, "stiff legged walk", or physical violence
o = ant lost before any interactions noted.

Recipient nest	Donor nest			
	29	34	65	64
29	----	+++++o	++++++	+o++++
34	++++o	----	-----	-----
65	+o++o+	------	-----	---
64	++++++	-----	----	----

would not be expected to differ, and neither would be identifiable as the offspring of a single queen.

These alternatives have been tested by the analysis of day and night (2300-2400 hrs) collections from nine nests in this large colony, arranged in three groups of three (A,B,C in Figure 8.4). The *Amylase* and *Esterase-1* genotype frequencies found in workers from these nests are shown in Table 8.11.

The data was tested for heterogeneity between various combinations of collections, using χ^2. Contingency tables ranged from 2 x 2 for the *Es-1* phenotypes of the day and night collections from a single nest, to 9 x 6, for overall heterogeneity of six phenotypes from each of nine nests. The results are summarized in Table 8.12. In various tests it was necessary to pool the rarest classes to obtain numbers large enough for analysis, and in some cases even this was not sufficient to meet the recommendations made by Evritt (1977). The tests which could not be done are indicated by a dash in the table.

Overall, the nine nests contained workers with different arrays of genotypes, both during the day and at night. During the day, *Amy* genotype frequencies were heterogeneous, but at night it was *Es-1* which showed heterogeneity. Contributing to this result is strong heterogeneity for *Amy* during the day within group B, and for *Es-1* within group A at night. The effect of within-group heterogeneity can be removed by pooling nests within a group. The among-group tests then reveal significant heterogeneity for both loci at night, but only marginal heterogeneity for the loci considered together, in the daytime collections.

Only one of the nests (488) contained different arrays of genotypes during the night and day, and this produced a difference between the night and day collections in the group to which it belongs (group B).

It appears from these results that this colony contains a number

Table 8.11. Phenotypes of workers from nine nests within a large colony of the red form of meat ants.

Allele notation abbreviated *e.g.*, a = *Amy*^{a} *etc.*, + = *Es-1* + phenotype, - = *Es-1* null phenotype.

N = night collections, D = day collections.

	Phenotype					
Nest	aa+	ac+	cc+	aa-	ac-	cc-
GROUP A						
482N	0	0	4	1	6	5
482D	5	1	2	0	3	5
483N	0	5	5	0	3	3
483D	0	5	4	0	3	4
484N	0	8	4	0	1	3
484D	2	2	2	0	3	7
GROUP B						
486N	0	2	1	0	8	5
486D	2	5	1	1	3	4
487N	1	4	1	1	3	6
487D	0	3	2	1	2	8
488N	1	1	1	0	5	8
488D	3	3	0	5	4	1
GROUP C						
492N	2	1	3	4	3	3
492D	0	1	5	3	7	0
493N	1	1	2	1	5	6
493D	0	2	0	1	10	3
494N	0	4	0	5	1	6
494D	0	7	3	4	0	2

Table 8.12. Analysis of heterogeneity among the genotype frequencies in samples from nine nests of the red form of meat ants (three groups A,B, and C, each comprising three nests).

* = significant heterogeneity at the 0.05 probability level;
** = significant heterogeneity at the 0.01 probability level;
\- = test not done due to small expectations.

Test	*Amylase*	*Esterase-1*	Combined
Among 9 nests - day	**		**
Among 9 nests - night		*	**
Among 3 nests in group A - day			
Among 3 nests in group A - night		**	
Among 3 nests in group B - day	**		-
Among 3 nests in group B - night			-
Among 3 nests in group C - day		*	**
Among 3 nests in group C - night			-
Among 3 groups - day			*
Among 3 groups - night	**	**	**
Day *vs* night, nest 482			-
Day *vs* night, nest 483			
Day *vs* night, nest 484			
Day *vs* night, nest 486			
Day *vs* night, nest 487			
Day *vs* night, nest 488	**		-
Day *vs* night, nest 492			-
Day *vs* night, nest 493			-
Day *vs* night, nest 494			-
Day *vs* night, Group A			
Day *vs* night, Group B	*		*
Day *vs* night, Group C			

of queens with varying genotypes, which produce cohorts of workers with different genotype frequencies. These groups of workers do not appear to mix as freely as their behaviour would suggest. Differences in genotype frequencies sometimes occur between nests which are quite close together, and groups of nests in widely separated parts of the colony also differ in the genotype frequencies of their workers, especially at night. This is consistent with a tendency of workers to return to their own nest at night, but homing behaviour does not appear to be universal. Even at night, most of the nests contain combinations of worker genotypes which cannot be produced by one singly-mated queen. Each nest can be visualized as a centre from which ants diffuse outwards, making smaller and smaller contributions to the population of nests located at increasing distances from their home nest. The amount of this outward movement seems to be much greater in the daytime than at night.

The colony is therefore not an amorphous, homogeneous unit as it appears at first sight. Rather, it has a complex organization in which structure is visible at several levels. These findings are consistent with information obtained from behavioural studies discussed in Chapter 2. A meat ant colony is characterized by a high level of social organization and division of labour, despite the fact that colony structure is very flexible, and that there are no clearly visible caste differences among workers.

8.5. Colony structure in the blue form

Previous sections have examined various aspects of the social behaviour of the red form of meat ants. Very little is known of the colony structure of the blue form, apart from the information in Greenslade (1970), and in Chapter 2.

Blue form colonies containing more than one queen can be detected by the appearance of both $Amy^{b}Amy^{b}$ and $Amy^{d}Amy^{d}$ workers, in the same way as for the red form (Section 8.2). The results are shown in Table 8.13. The number of recognisable two-queen colonies is less than expected, although the sample sizes are too small to state this with complete confidence. The results suggest that some, but by no means all, blue form colonies contain more than one queen.

The detailed map of blue form colonies presented in Figure 2.6 allows the direct examination of nests and colonies whose relationships are known. Four colonies (marked A,B,C,D in Figure 2.6) were chosen, and the location of nests in each was carefully marked during the afternoon of Thursday 18th November, 1976. Ants were collected from these holes between 0600 and 0630 hrs, the next morning, before they began to move about on the surface. The *Amylase* genotypes of these workers are shown in Table 8.14.

Colonies A, C, and D yielded almost exclusively $Amy^{b}Amy^{b}$ workers. The only exceptions are a single heterozygous worker in nest 497 (colony A), and three more in nest 503 (colony C). Colony B however, shows segregation among the workers from all three holes sampled. Colonies A and B are adjacent, and have holes separated by less than 15 metres. Nevertheless, their workers are inter-hostile, and have different *Amylase* genotype frequencies. The single heterozygous worker in nest 497 could have come in from colony B, or it could indicate the existence of a second queen somewhere in colony A. The three heterozygotes found among

Table 8.13. Frequency of blue form nests containing workers of two different homozygous genotypes for the *Amylase* locus.

Exp. = expected number of such nests calculated on the assumption that every nest contains two queens (k x N).

Obs. = Observed number.

Population	Number of nests (N)	k	Exp.	Obs.
Hallett Cove	3	0.2077	0.623	1
Eyre Penin.	9	0.1462	1.316	0
Flinders Ra.	10	0.2188	2.188	1
Dublin	17	0.2133	3.626	1
Eudunda	17	0.2174	3.696	1
Morgan	18	0.2186	3.935	1
Snowtown	4	0.1754	0.702	1
			16.085	6

Table 8.14. Genotypes of workers from 15 nests of the blue form of meat ants. Colonies A and B occupy adjacent territories, as do colonies C and D.

Nest	Colony	Workers	
		$Amy^{b}Amy^{b}$	$Amy^{b}Amy^{d}$
495	A	12	0
496		12	0
497		15	1
498		13	0
499	B	9	4
500		5	9
501		10	4
502	C	12	0
503		12	3
504		12	0
505		12	0
506		12	0
507	D	12	0
508		12	0
509		11	0

60 homozygotes in colony C may also indicate the presence of more than one queen. The three nests making up colony B are homogeneous in genotype frequencies (χ^2_2 = 4.58, 0.2>P>0.1), so it is likely that the workers collected from these nests were all produced by a single queen.

Several conclusions can be drawn from the results presented in this section -

a) territorial boundaries in the blue form are sometimes marked by sudden changes in the genotype frequencies of workers, as they are in the red form;

b) a multi-hole colony may contain only a single queen, or it may have more than one;

c) it follows from b) that workers which developed in one hole do not necessarily form a permanent attachment to it, but may spend nights or longer periods in some other hole.

Obviously a larger scale survey of blue form colonies is needed to confirm these findings. It would be of particular interest to sample from colonies containing many (say, 50) nests, as I have done with the red form, so that the results could be compared with those from the relatively simple situation described here.

Chapters 4 to 8 have examined a number of aspects of genetic variation in meat ants, and several different conclusions have been reached. The arguments in each case have been based on findings from one or two loci. The next two Chapters extend this approach, to the simultaneous consideration of a larger number of loci, using the parameters of average heterozygosity and genetic distance.

CHAPTER 9

LEVELS OF PROTEIN POLYMORPHISM IN MEAT ANTS AND OTHER ORGANISMS

9.1. Population genetics of sex linkage and arrhenotoky

Meat ants, like all other Hymenoptera, have haploid males and diploid females. This system ("arrhenotoky") therefore resembles the situation for sex-linked loci in diploid organisms, and it is possible to picture Hymenoptera as species in which every locus is sex-linked. It is therefore of interest to examine theoretical studies of the population genetics of sex-linked loci, to see whether their findings can be applied to the Hymenoptera.

Haldane (1924, 1926) examined the population genetics of sex-linked loci under a variety of selective systems. With no selection, the genotype frequencies in males and females were shown to converge in an oscillatory manner to their equilibrium values. Selection against a sex-linked recessive gene was found to proceed much more quickly than for an autosomal one, because of its exposure in males every generation. Haldane (1932) also showed that selection could maintain a balanced polymorphism at a sex linked locus, and pointed out that in "the social Hymenoptera, all genes are sex-linked. This fact may have accelerated their evolution".

The conditions for a selectively balanced polymorphism at a sex-linked locus were examined in more detail by Bennett (1957, 1958) and Sprott (1957). The conclusions reached were that, unlike the autosomal situation, heterozygote advantage is neither necessary nor sufficient for stability. In particular, selection favouring different alleles in males and females could result in a balanced polymorphism, and selection against one allele in males could offset heterozygote advantage in females, and lead to the disappearance of this allele from

the population. This result is also described by Mandel (1959), Haldane and Jayakar (1964), and Kirkman (1966).

Li (1967a,b) assigned fitness values to the genotypes of males and females thus:

	Females			Males	
Genotype	AA	Aa	aa	A	a
Fitness	W_1	W_2	W_3	V_1	V_3

The conditions for a stable polymorphism derived by Li and earlier authors can then be summarized as

$$V_1W_1 \quad < \quad \tfrac{1}{2}W_2\,(V_1 + V_3) \quad > \quad V_3W_3$$

If these conditions apply, gene frequencies inevitably converge to an equilibrium point and remain there once it is reached (Cannings, 1967; Palm, 1974). **Stable polymorphisms** can also be attained in the case of multiple alleles (Cannings, 1968; Nagylaki, 1977a) or overlapping generations with continuous selection (Nagylaki, 1975). The conditions for stable polymorphism at a di-allelic sex linked locus have been summarized more recently by Cavalli-Sforza and Bodmer (1971), Jacquard (1974), and Edwards (1977).

The above expression for the conditions for a stable polymorphism at a sex linked locus requires a balance between the fitness values of five genotypes instead of three, as in the autosomal case. At first sight this might suggest that selectively balanced polymorphism under sex linkage or arrhenotoky would be unlikely.* Indeed, several authors have predicted that arrhenotokous insects would have significantly reduced stores of genetic variation (*e.g.*, White, 1954; Suomalainen,

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* In kangaroos, the inactivation of the paternally derived X-chromosome in females is expected to make selectively balanced polymorphism at X-linked loci even less likely (Cooper, 1975). However, the small amount of data presently available does not appear to support this prediction (Cooper *et al.*, 1976).

1962; Askew, 1968; Slobodchikoff and Daly, 1971; Wilson, 1971.

Crozier (1970a) simulated selection at sex-linked and autosomal loci by assigning fitness values to genotypes at random. Out of 200 sets of fitnesses tried, 68 gave stable sex-linked polymorphisms, compared with 75 for an autosomal locus, results which are not significantly different ($\chi^2_1 = 0.53$; $P \simeq 0.5$). In this simulation, sex-linked loci did not have a substantially reduced frequency of selectively balanced polymorphisms. The use of the random number generator of a computer to assign fitness values is said to produce a similar result (Crozier, 1975).

I have repeated this experiment using 10,000 sets of fitnesses assigned by the library computer program RANF, which generates random numbers between zero and one. The result was 3304 stable autosomal polymorphisms, based on the criterion that the heterozygote is superior to both homozygotes, and 3101 sex linked polymorphisms, from the criterion of Li (1967). This result is very similar to that of Crozier (1970a), but the larger number of trials allows the difference to become statistically significant ($\chi^2_1 = 9.47$).

It may be unrealistic to allow the fitness of genotypes to vary from zero to one in this way, since in real situations, large selective differences are probably uncommon. Therefore, I have examined the effect of varying the range of fitness values, by setting the upper limit of fitness at one, and varying the lower limit between zero and 0.9. Manipulation of the range of the random number sequence has been done in two ways. In the first instance, any number lower than the required lower limit of fitness was simply discarded, and another number used, until 10,000 sets of numbers in the required range had been found. Figure 9.1 shows the result. Raising the lower limit of fitness does not have a clear effect on the number of autosomal polymorphisms, but causes a decline in the number of X-linked polymorphisms.

Figure 9.1.

Relationship between frequency of polymorphism and fitness range, based on sequences of random fitness values.

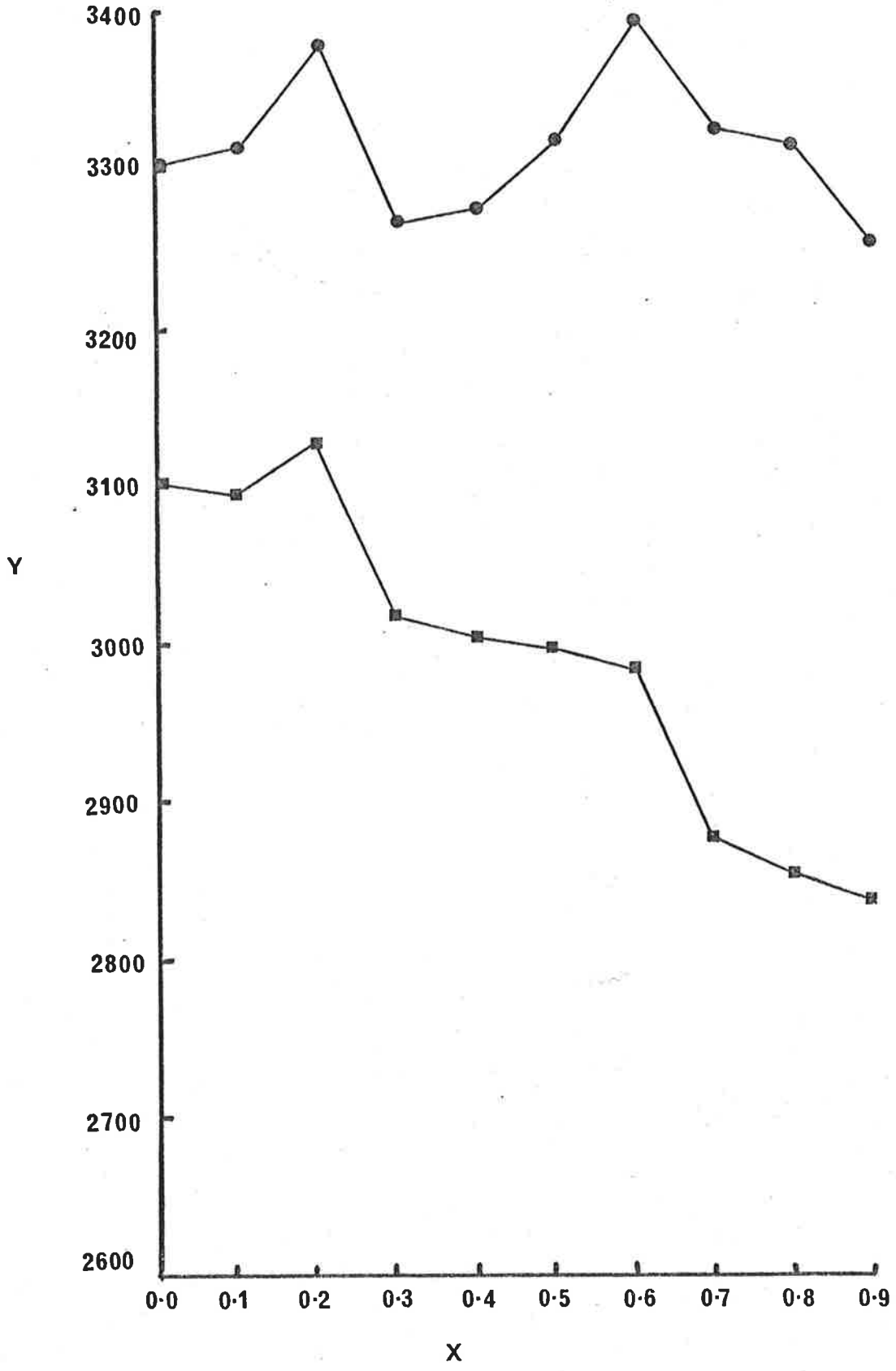
Fitness values chosen at random, and truncated to fit into the required range from x to one.

X = lower limit of fitness.

Y = number of polymorphisms successfully established from a sample of 10,000.

● = autosomal polymorphisms.

■ = X-linked polymorphisms.


3400
3300
3200
3100
3000
2900
2800
2700
2600
Y
0·0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
X

However, selection of random numbers in this way means that the result for each fitness range is based on a different set of random values. The irregularities in the lines in Figure 9.1 can be attributed to chance differences between these different sets of fitnesses. This effect can be removed by using a single series of random numbers for each fitness range. If the lower limit of fitness required is D, and F is a random number between zero and one, then W = 1 - F(1 - D) is a random number between D and one. A single series of random numbers can then be compressed into the range from D to one, for varying values of D. The number of autosomal polymorphisms produced by fitnesses chosen in this way is constant as D changes, by the number of X-linked polymorphisms declines steadily (Figure 9.2).

Thus, if there is a real reduction in the frequency of selectively balanced polymorphisms for sex-linked loci, then this reduction may increase in magnitude as the range of fitnesses narrows.

However, this simulation, like that of Crozier's (1970a), assigns fitnesses to genotypes in males and females independently. Hartl (1971) chose an alternative assumption, that the fitness of a homozygous female is the same as that of the corresponding hemizygous male (in Li's symbolism $W_1 = V_1$ and $W_3 = V_3$). His finding was that there would be "fewer polymorphisms maintained by overdominance in a male haploid population than in a comparable diplodiploid population". He also showed that if a beneficial mutation appeared, selection in favour of it would be very strong, and substitution would take place faster than in a diploid species (Hartl, 1971, 1972). This is in agreement with the earlier findings of Haldane (1932).

The general question arises then, of how the fitnesses of genotypes in males and females are related, if at all, and specifically, to what extent loci are sex limited in their effects on fitness.

Many of the authors previously cited agree that loci which

Figure 9.2.

Relationship between frequency of polymorphism and fitness range, based on sequences of random fitness values.

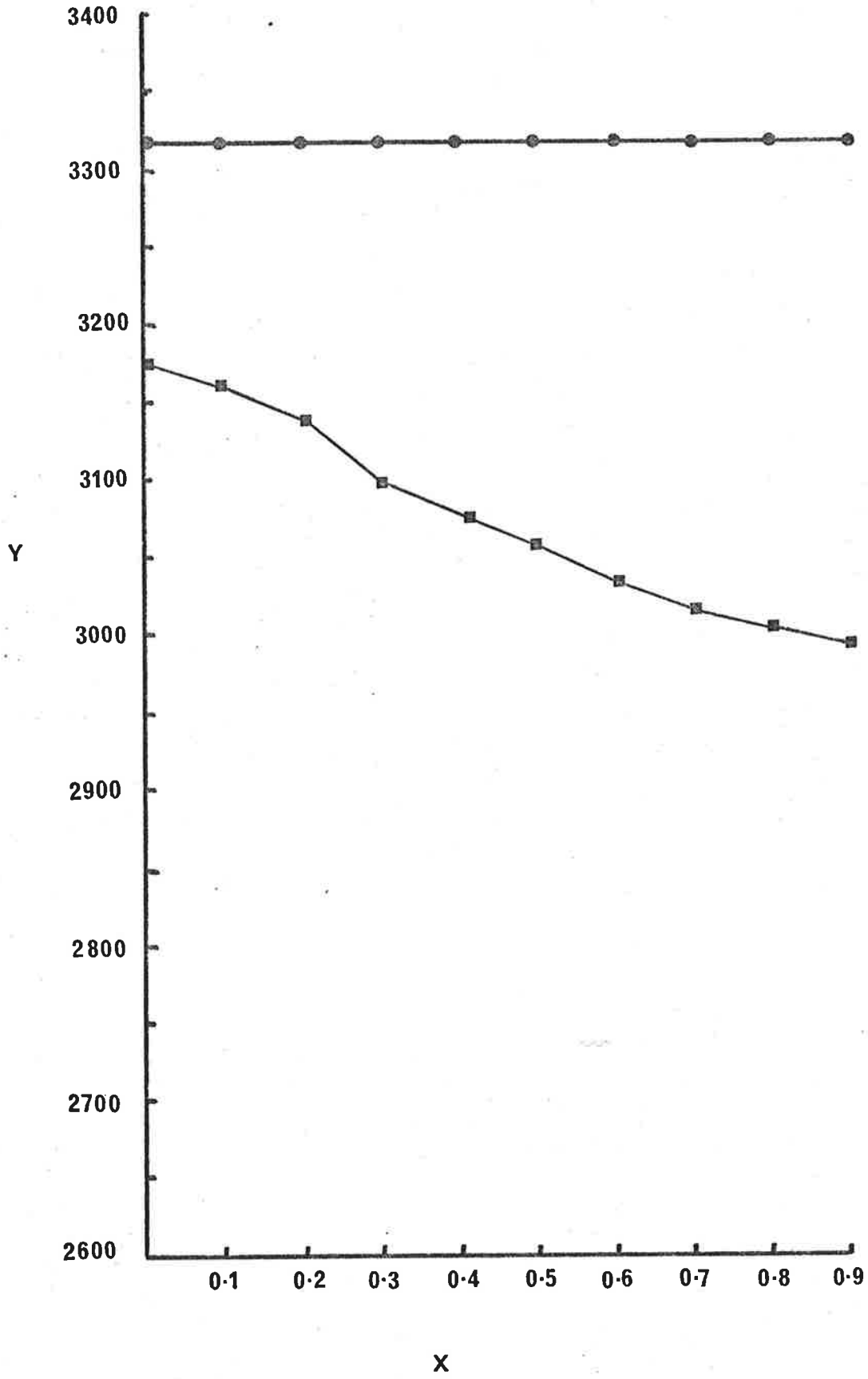
Fitness values chosen at random and compressed to fit into the required range from x to one.

X = lower limit of fitness.

Y = number of polymorphisms successfully established from a sample of 10,000.

● = autosomal polymorphisms.

■ = X-linked polymorphisms.


3400
3300
3200
3100
3000
2900
2800
2700
2600
Y
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
X

affect only females will allow selectively balanced polymorphism more often than those which are not sex-limited (*e.g.*, White, 1954; Crozier, 1970a; Wilson, 1971). The X-chromosome of *Drosophila melanogaster* has been shown to carry lethal and deleterious genes which are limited in their effect to females (Kerr and Kerr, 1952; Drescher, 1964). However, this result, as for all the information derived from sex-linked loci, must be interpreted with caution. The mathematical treatment of selection in an arrhenotokous organism may be the same as for sex-linkage, but in terms of regulation and physiology, the results may be very different (Crozier, 1970a).

The degree of sex-limitation of deleterious genes has been measured in some species of Hymenoptera, and has been found to be considerable (*e.g.*, Kerr, 1976; Crozier 1975, 1976b). However, the assumption of Hartl (1971) that the fitnesses of genotypes in males and females are completely correlated, and that of Crozier (1975) that they are completely independent, are both extreme views. If a large number of loci are considered, most will probably be found to lie between these extremes. Even if there is a proportion of loci which are sex-limited this need not have a marked effect on the genetic load experienced by a species (Crozier, 1976a).

To summarize, if there is a reduction in the level of polymorphism in Hymenoptera, the amount of this reduction will depend not only on the formulae derived from the theory of sex linkage, but also on the extent of sex limitation, the relationships between the fitnesses of males and females, and the range in which fitness values lie. It is also worth remembering that types of selection other than heterozygous advantage, such as density or frequency dependent selection, can act to maintain polymorphisms in haplodiploids as they can in other organisms (Crozier, 1970a). Selection at the colony level may also contribute to maintaining genetic variation in social Hymenoptera under

some circumstances (Crozier and Consul, 1974, 1976). Equilibrium between mutation and selection is also possible, and the gene frequencies resulting from this process have been derived by Nagylaki (1977b).

While the importance of these factors remains unknown it is not possible to make clear predictions about the levels of genetic variation to be expected in Hymenoptera. It is clear though, that more factual data are needed, concerning both the levels of variation actually found, and the nature and functioning of sex limitation. The remainder of this chapter examines the first of these areas.

9.2. Levels of protein polymorphism in meat ants.

The amount of polymorphism at protein and enzyme loci was measured in four populations of meat ants. The populations used were those of the red, black, and blue forms from Morgan, and the red form from Arthurton (see Chapter 3 for details). The frequencies of alleles at 15 loci were calculated, and are presented in Table 9.1. Details of the loci used and the phenotypes involved have been described previously.

The sample sizes shown for the number of alleles sampled per locus have been derived from three times the number of nests, based on the assumption of one singly mated queen per nest. Evidence from several sources shows that this may be incorrect in some cases, and that some nests contain more than one queen. Therefore, the sample sizes shown are probably underestimates of the number of genes actually involved. A sample of 45 genes gives at least a 90% probability of detecting an allele whose frequency is 0.05 (Mather, 1957). Therefore, if a low level of genetic variation is observed in these data, it cannot be attributed in a major way to the use of samples with a minimum size of 45. Furthermore, the number of workers sampled per nest in these populations has a minimum of seven and a mean of 9.5, so there is little chance that segregating nests have been mis-classified as monomorphic.

Twelve of the 15 loci do not show variation in any nest in these populations, and the loci which do vary are *Esterase-1*, *Esterase-2* and *Amylase*. Within each population, either one or two loci are polymorphic, giving a proportion of polymorphic loci of 0.067 or 0.133, with a weighted mean of 0.118. The criterion usually used to decide whether a locus is "polymorphic" is that the most common allele has a frequency of less than 0.99. Thus, in samples such as these, with less than 100 genes, any genetic variation at all is counted as a

Table 9.1. Allele frequencies and levels of genetic variation in four populations of meat ants.

Allele	Locality and colour form			
	Morgan Red	Morgan Black	Morgan Blue	Arthurton Red
Gdh^a	1.0	1.0	1.0	1.0
Sod^a	1.0	1.0	1.0	1.0
Me^a	1.0	1.0	1.0	1.0
Mdh-1^a	1.0	1.0	1.0	1.0
Mdh-2^a	1.0	1.0	1.0	1.0
Lap^a	1.0	1.0	1.0	1.0
Ldh^a	1.0	1.0	1.0	1.0
Es-1^o	0.833	0.924	1.0	0.625
Es-1$^+$	0.167	0.076	0.0	0.375
Es-2^o	0.0	0.0	0.330	0.0
Es-2$^+$	1.0	1.0	0.670	1.0
Es-3^a	1.0	1.0	1.0	1.0
Amy^a	0.233	0.0	0.0	0.0
Amy^b	0.0	0.0	0.509	0.982
Amy^c	0.767	1.0	0.0	0.018
Amy^d	0.0	0.0	0.491	0.0
P-1^a	1.0	1.0	1.0	1.0
P-2^a	1.0	1.0	1.0	1.0
G6pd^a	1.0	1.0	1.0	1.0
Ao^a	1.0	1.0	1.0	1.0
Number of genes sampled per locus	48	45	51	57
Proportion of loci polymorphic	0.133	0.067	0.133	0.133
Average heterozygosity per locus ($\bar{H}$)	0.0424	0.0094	0.0628	0.0336
S.E. of $\bar{H}$	0.0298	0.0096	0.0437	0.0317

polymorphism.

The average proportion of loci at which an individual queen is heterozygous ($\bar{H}$) in each population ranges from 0.0094 to 0.0628, with a weighted mean of 0.0377. These are expected levels of heterozygosity - observed values cannot be obtained since heterozygotes for *Es-1* and *Es-2* cannot be detected. Also, it is assumed that gene frequencies in males and females are equal, and males obviously cannot be heterozygous. Nevertheless, it is clear that on average a female meat ant is likely to be heterozygous at approximately 0.038 of the loci producing the enzymes and proteins examined. This level of heterozygosity is substantially lower than that commonly found in insects (see Section 9.3.).

Apart from the average level of heterozygosity across loci, it is also possible to compute the frequency of heterozygotes for each locus considered separately. With two alleles, this obviously has a maximum of 0.5. The frequency distribution of single locus heterozygote frequencies in meat ants is shown in Figure 9.3, for all four populations pooled. Most loci have almost zero heterozygosity, and a small minority approach 0.5. This is very similar to the result in humans, *Mus*, and *Drosophila*, and also to the distribution predicted by the neutral allele model (Nei *et al.*, 1976; and included references). The figure also shows the commonly observed secondary peak near 0.5. This could result from the pooling of loci which have varying numbers of alleles (Ewens and Gillespie, 1974; Stewart, 1976), although none of the meat ant populations considered here have more than two alleles at any locus.

Comparison of the distribution of single locus heterozygosity in meat ants with those of other species reveals only one conspicuous difference. The frequency of loci which have little or no heterozygosity

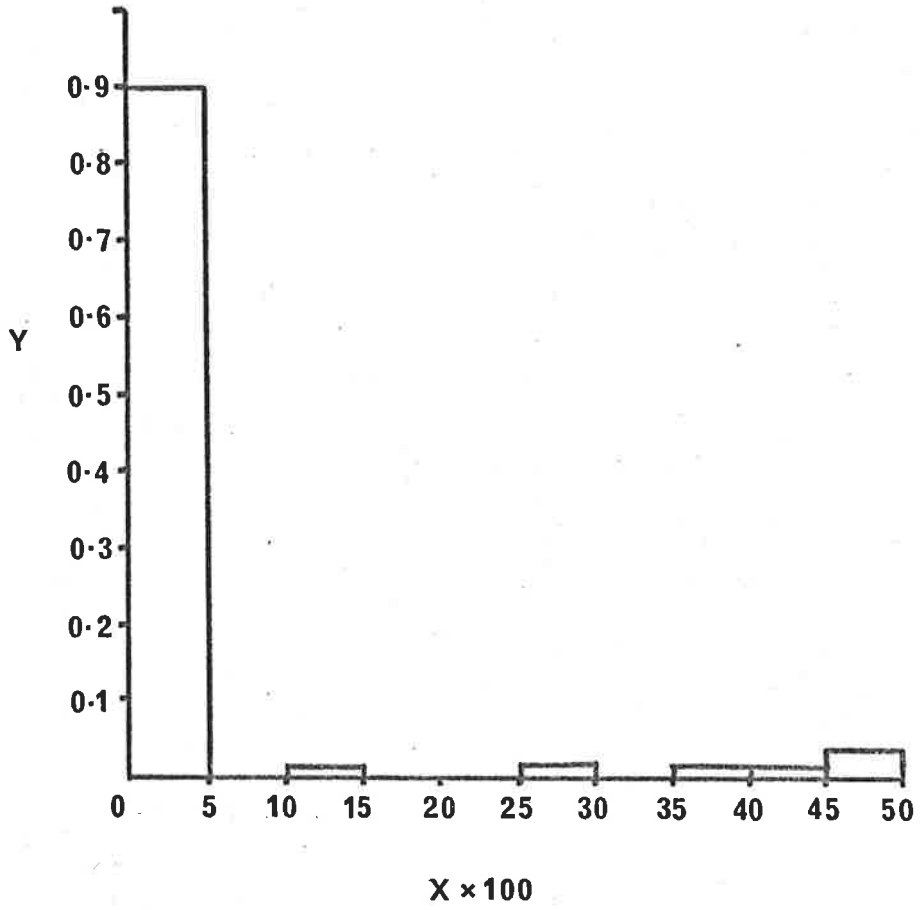


Figure 9.3

The distribution of single-locus heterozygosity in meat ants (4 populations pooled).

X = expected frequency of heterozygotes at individual loci.

Y = frequency of loci.

is much higher ~ 0.9, compared with 0.75 or less in the other organisms considered by Nei *et al.*, (1976). This is another aspect of the low overall level of heterozygosity found in meat ants.

The next section examines the results of other surveys of genetic variation in Hymenoptera, and compares their findings with the data obtained from meat ants.

9.3. Levels of protein polymorphism in other Hymenoptera.

It has been known for some time that species of Hymenoptera can show genetic variation. Indeed, several species have come to be used routinely in genetical research. The honeybee *Apis mellifera* has been investigated genetically for many years, and a large number of mutant genes affecting morphology, disease resistance and behaviour are known (reviewed by Kerr, 1974; Rothenbuhler, 1975). Two parasitoid species, *Habrobracon juglandis* and *Mormionella vitripennis* are also the subjects of an extensive literature on genetic variation (reviewed by Cassidy, 1975). Less is known about other species, but genetic variation has been reported in *Aphytis mytilaspidus* (Rössler and DeBach, 1975), and a number of other species (see Crozier, 1968b). Also, several chromosomal polymorphisms are known, effecting both the number of chromosomes and their morphology (reviewed by Crozier, 1975).

The arrhenotokous mites *Tetranychus urticae* and *T. pacificus* have also been the subjects of genetical research, and genetic variation is known in morphology, pesticide resistance, and photoperiod response (Boudreaux, 1963; Helle, 1968; Ballantyne, 1969).

Allozyme polymorphisms have also been reported in a variety of species of Hymenoptera (see previous Chapters). Crozier (1970a) noted that quantitative estimates of the amounts of genetic variation in Hymenoptera had not been made. Since that time, several independent investigators have examined this question.

Snyder (1974) studied three species of bees which vary in their levels of social behaviour. He surveyed 24 loci in *Lasiglossum zephyrum*, 13 in *Augochlora pura*, and 12 in *Bombus americanorum*, without finding any genetic variation at all. The selection of enzymes used included several peptidases and esterases, enzymes which commonly vary in other organisms, and the collections used range from 20 to 130 females. The lack of any genetic variation at all in these enzymes is perhaps

surprising, but the conclusion seems clear that these three species appear to have very low levels of enzyme polymorphism.

Brückner (1974) also failed to find any variation at three loci in the honeybee (referred to as *Apis mellifica*). A total of 14 hives were examined, including four subspecies. These small sample sizes reduce the probability of finding variation, and it is interesting to note that Brückner found "Esterase" to be monomorphic, while Mestriner (1969; Mestriner and Contel, 1972) reported a clearly resolved esterase polymorphism. This difference may be a real one, since the hives studied came from Germany and Brazil respectively, or as suggested by Hung and Vinson (1977b), it could reflect differences in electrophoretic techniques.

The work of Pamilo *et al.*, (1975) in the *Formica rufa* group of ants also suffers from small sample sizes. The maximum number of nests examined for any locus was nine, for *Mdh* in *F. polyctena*, and much of the data was based on just one nest per species. Thus, it is not surprising that little variation was found. For the α-*Gpdh* locus in *F. lugubris*, 58 workers and two queens were sampled from four nests. One nest contained only one heterozygous worker out of 46 tested. Pamilo *et al.* apparently interpret this as the existence of a rare allele with a frequency of 0.0083 (from 1/120). The existence of rare heterozygotes within a nest is surprising, and the problems of estimation gene frequencies directly from workers have been pointed out previously. The result for *Mdh* in *F. exsecta* is also surprising - in one nest, the workers typed apparently included 21 identical homozygotes, one differing homozygote and two heterozygotes. These results may be an indication of polygyny, since Pamilo *et al.* state that some species in this group may have up to several hundred queens per nest. These problems make it difficult to assess whether the apparent lack of variation reported by Pamilo *et al.* is a general phenomenon in *Formica*, but their results are

consistent with the suggestion that the level of genetic variation in these species may be low.

Metcalf *et al.* (1975) surveyed large samples of seven species of solitary bees and wasps for allelic variation at 12-19 loci. Many of these loci showed allelic variation in one or more species, and the proportion of loci that were polymorphic ranged from 0.13 to 0.37 per species (mean = 0.266). The level of heterozygosity ranged from 0.038 to 0.078, with a mean of 0.061. These figures were found to be significantly lower than the corresponding values in a range of diploid species.

Similar observations have been made in seven species of solitary and social wasps in the genera *Megachile*, *Nomia*, *Opius*, and *Polistes* (Lester, as cited by Selander, 1976; Lester and Selander, 1977). The mean number of loci surveyed per species was 15, and the mean proportion of loci heterozygous per individual was 0.062. One of these species, *Polistes apachus*, has the highest level of heterozygosity yet recorded for a Hymenoptera species, 0.112. This appears to have been caused by human interference with its habitat, and by introgression of genes from *P. bellicosus* by hybridization.

P. S. Ward (pers. comm.) has surveyed 22 protein and enzyme loci in three species of Australian ants of the *Rhytidoponera impressa* group, and found an average level of heterozygosity of approximately 0.035±0.030. This figure is based on six populations of *R. chalybaea*, two of *R. cyrus*, and one of an unnamed species. The level of heterozygosity in *Rhytidoponera* is therefore remarkably similar to that in meat ants.

The results of all these studies are summarized in Table 9.2. Excluding *Polistes apachus*, and excluding studies of less than ten loci, the data includes 22 species, which have an unweighted mean heterozygosity of 0.044±0.022 (counting the three colour forms of meat ants as separate species).

Table 9.2. Levels of heterozygosity observed in Hymenoptera. References:- 1 = Metcalf *et al.*, 1975; 2 = Lester and Selander, 1977; 3 = Snyder, 1974; 4 = Brückner, 1974; 5 = Pamilo *et al.*, 1975; 6 = this study; 7 = P.S. Ward, pers. comm.

Species	Type of species	no. of loci	$\bar{H}$ ± S.E.	Ref.
Stictia carolina	Solitary wasp	17	0.056±0.029	1
Chalybion californicum	Solitary wasp	16	0.073±0.036	1
Sceliphron caementarium	Solitary wasp	12	0.078±0.055	1
Scolia dubia	Solitary wasp	15	0.051±0.032	1
Trypargilum politum	Solitary wasp	19	0.059±0.028	1
Nomia heteropoda	Solitary bee	15	0.070±0.048	1
Savastra obliqua	Solitary bee	16	0.038±0.021	1
Opius juglandis	Solitary wasp	13	0.043±0.006	2
Megachile pacifica	Solitary wasp	14-19	0.033±0.011	2
Nomia melanderi	Solitary bee	13	0.041±0.010	2
Polistes annularis	Social wasp	15	0.057±0.011	2
Polistes apachus	Social wasp	13	0.112±0.021	2
Polistes bellicosus	Social wasp	13	0.071±0.025	2
Polistes exclamans	Social wasp	16	0.061±0.012	2
Lasioglossum zephyrum	Social bee	24	0.0	3
Augochlora pura	Solitary bee	13	0.0	3
Bombus americanorum	Social bee	12	0.0	3
Apis mellifera	Social bee	3	0.0	4
Formica (8 species)	Ants	5-9	low?	5
Iridomyrmex purpureus				
Red form	Ant	15	0.039±0.031	6
Blue form	Ant	15	0.064±0.044	6
Black form	Ant	15	0.010±0.010	6
Rhytidoponera chalybaea	Ant	22	0.031±0.035	7
Rhytidoponera cyrus	Ant	22	0.046±0.024	7
Rhytidoponera sp.	Ant	22	0.036±0.024	7

A large body of data from surveys of other organisms is now available, and has recently been reviewed by Lewontin (1974), Nei (1975), Powell (1975) and Selander (1976). Using data summarized by Powell (1975), the results from Hymenoptera are compared with those from other groups in Table 9.3. On this basis, Hymenoptera are significantly less variable than *Drosophila*, other insects, and non-insect invertebrates, and have a level of heterozygosity comparable to that found in the sample of vertebrates.

Table 9.3. Comparison of levels of heterozygosity in Hymenoptera with that in other groups. The Hymenoptera data come from 22 species, with $\bar{H}$ = 0.044±0.022. (See Table 9.2 for data).

Data for other groups from Powell (1975).

Group	Number of species	$\bar{H}$ ± SE	t	Prob.
Drosophila	38	0.157±0.009	23.00	<0.001
Other insects	7	0.170±0.027	11.22	<0.001
Non-insect invertebrates	13	0.102±0.021	7.76	<0.001
Vertebrates	71	0.050±0.004	1.27	0.2-0.4

9.4. Discussion

An apparent difference between two organisms in their level of heterozygosity may not necessarily reflect the existence of real variation. Gillespie and Kojima (1968) found that on average enzymes involved in energy production showed less genetic variation than "non-specific" enzymes. Johnson (1974) classified enzymes differently, and found that "variable substrate" enzymes were the most variable, "non regulatory" enzymes were the least variable and "regulatory" enzymes were intermediate. Powell (1975) showed that this trend occurred in almost every group of animals studied. Also, the amount of variation shown by a particular enzyme may depend on the number of subunits in an active molecule (Ward, 1977), or on the size of these subunits (Koehn and Eanes, 1977). The particular types of enzymes used in a survey of heterozygosity could therefore have an influence on the results. Spurious differences in heterozygosity levels could also be produced by variations in the efficiency of electrophoresis. Johnson (1977) reviews the evidence that variation in mobility for some enzymes may be evident under some electrophoresis conditions but not others.

The surveys of Hymenoptera shown in Table 9.2 are based on widely varying selections of loci, and still give generally consistent results. Also, in Lester's surveys, the reduction in heterozygosity from *Drosophila* to wasps was found to be distributed fairly evenly over all loci (cited by Selander, 1976). These observations, together with the fact that low levels of heterozygosity in Hymenoptera have been observed independently at least five times, strongly suggest that the reduction is a real one, and does not depend heavily on the choice of enzymes used, or on the visual acuity of the experimenter. If this is so, it is legitimate to examine some of the factors which are thought to influence levels of heterozygosity, to determine whether they can account for the low level

in Hymenoptera generally, and in meat ants in particular.

Environmental heterogeneity

Many theoretical and empirical models predict an association between environmental heterogeneity and genetic variation (reviewed by Hedrick *et al.*, 1976). The so-called "niche-variation" hypothesis (Van Valen, 1965) states that an organism which occupies a heterogeneous environment and has a wide ecological niche, gains it tolerance and flexibility by carrying a large number of genetic polymorphisms. Conversely, a species which has a narrow niche, or whose members collectively experience a narrow range of environmental conditions, has a single optimum phenotype, and its members are generally homozygous for the genes contributing to it. Levins (1968) considered the stability of the environment in terms of "environmental grain". If the physical environment of an organism is "coarse-grained", each individual experiences only a restricted range of conditions, which may differ considerably from that experienced by another individual. This is seen as favouring genetic polymorphism. In a "fine-grained" environment, each individual experiences the same range of conditions, and polymorphism is less likely. Theoretical support for these ideas is provided by Gillespie (1974; Gillespie and Langley, 1974).

The occupation of a narrow ecological niche or a very homogeneous environmenthas been suggested as causes of low levels of heterozygosity in *Drosophila busckii* (Prakash, 1973), the mole rat *Spalax ehrenbergi* (Nevo and Shaw, 1972), and the pocket gopher *Thomomys talpoides* (Nevo *et al.*, 1974). The level of heterozygosity in four species of amphibians is positively correlated with their levels of environmental heterogeneity in terms of climatic factors (Nevo, 1976), and *Bufo viridis*, which experiences a very variable environment, has a very high level of heterozygosity (Dessauer *et al.*, 1975; Nevo *et al.*, 1975). Bryant (1974a,b) also found positive correlations between the amount of genetic variation

in a number of species and the temporal stability of their environments, as measured by weather records, and *Drosophila* populations maintained in temporally or spatially variable environments appear to maintain more genetic variation than those kept in uniform ones (Powell, 1971, McDonald and Ayala, 1974). Any tendency for spatial heterogeneity in the environment to encourage genetic polymorphism, is likely to be reinforced if each member of the species concerned chooses to occupy the particular habitat where it is most fit (*e.g.*, Taylor, 1976).

When considering marine habitats, Bretsky and Lorenz (1969, 1970) suggested that a stable, predictable environment would favour reduced levels of genetic variation. However, both the deep sea (Gooch and Schopf, 1972), and tropical reefs (Ayala *et al.*, 1973; Valentine *et al.*, 1973; Campbell *et al.*, 1975) support organisms which contain quite high levels of heterozygosity.

Valentine (1976; Valentine and Ayala, 1974, 1976) considers instead that the level of genetic variation in marine organisms is influenced by the stability of its food supply (the "trophic resource stability model"). A supply of food which is unpredictable in amount and composition is thought to favour a generalized "all purpose" genotype, and a reduced level of heterozygosity. This hypothesis seems to be supported by results from a large number of marine organisms.

However, like most models seeking to relate environmental and genetic heterogeneity, this suggestion is difficult to test. Even when an attempt is made to identify and measure the environmental features thought relevant to an organism, their relationship to genetic variation may be inconclusive (*e.g.*, Sabath, 1974). Also, it may be unrealistic to expect different species to respond to environmental heterogeneity in the same way. Hedrick *et al.*, (1976) reach the inevitable conclusion that although many hypotheses predict a correlation between levels of genetic and environmental heterogeneity, there is little or no unequivocal

evidence with which to assess their relative merits.

The uncertainties involved in the testing of these models make it difficult to know how to apply them to meat ants. The red form of *Iridomyrmex purpureus* was shown in Chapter 2 to be ecologically very generalized and omnivorous, and there is no reason to suppose that the other colour forms are any less so. However, they live underground, and their nests provide some protection from fluctuations in the external environment (Ettershank, 1971). The stability of both the subterranean habitat (Nevo, 1976) and conditions in a nest or hive (Brückner, 1974; Snyder, 1974) have been suggested as favouring reduced genetic variation.

Also, during the lifetime of a single meat ant queen, many variations will occur in the environment around the nest, including seasonal and long term changes in climate, vegetation, and the abundance of other species of animals. Thus, the optimum "strategy" may be to produce workers with a uniform "all purpose" genotype, capable of exploiting all aspects of this temporally changing environment. Thus, at least two different forces could act together to produce a low level of genetic variation in meat ants, through a relationship with environmental heterogeneity, although the diversity of models available makes it almost inevitable that some of them will be relevant to any particular situation.

Mobility and size

Selander and Kaufman (1973) argue that a large animal (such as a vertebrate) is likely to be more mobile than a small one (such as an insect), and therefore more likely to experience its environment as fine grained. A single optimal genotype is therefore favoured. The relatively coarse-grained environment of a small, less mobile animal is considered to favour genetic polymorphism. Reinforcing this effect is size itself. A large animal has more ability to achieve physiological homeostasis than a small one, and this buffering of environmental fluc-

tuations may reduce the level of heterozygosity. However, others (*e.g.*, Johnson, 1974) would argue that this homeostasis is itself partly achieved by heterozygosity, so the argument is not completely unambiguous.

Selander and Kaufman's (1973) argument seems to be supported by the general trends in the large body of data which they present. Despite this, the figures presented by Powell (1975) reveal that species within the single genus *Drosophila* have heterozygosity values ranging from 0.044 to 0.242 - a range greater than that for all vertebrates (0 - 0.135). This shows that there are factors effecting heterozygosity at the individual species level, which may be at least as important as any forces associated with size or mobility on a more general scale.

Individually, meat ants are not large. However, it seems likely that the unit of selection in social insects is the colony, which in some ways resembles a much larger animal, with some ability to achieve homeostasis of conditions within the nest. Large size, in terms of the colony unit, is only achieved at the expense of mobility, so the correlation between these two factors in Hymenoptera becomes a *negative* one. This argues against the usefulness of the Selander/Kaufman hypothesis in accounting for the low levels of genetic variation in these organisms.

Age of species

Soulé and others have also found results which are inconsistent with Van Valen's (1965) niche-variation hypothesis. The types of variation studied include morphological variation in birds (Soulé and Stewart, 1970) and lizards (Soulé, 1972), and electrophoretic variation in lizards (Soulé and Yang, 1973) and fish (Somero and Soulé, 1974). These results form the basis of the "time-divergence" hypothesis (reviewed by Soulé, 1976).

According to this hypothesis,when a species is first formed, or first colonises a new island, it is subject to strong directional selection,

which erodes genetic variation. Later, when the species or population faces competition from an increasing number of other species, and when a stable community is developed, stabilizing selection becomes important. This acts mainly through the accumulation of heterotic polymorphisms. The main prediction from this hypothesis is that species which have occupied ecologically stable, diverse habitats for long periods of time, and which have large population sizes, should contain high levels of genetic variation. This model therefore agrees with the trophic resource stability model of Valentine (1976), in predicting high levels of variation in deep sea animals.

The main problem with this hypothesis is that it refers to many variables which are very difficult to measure, such as population size, evolutionary time, and ecological parameters. It will therefore be very difficult to test, as Soulé himself admits. Meat ants seem to fit the requirements of a slowly evolving, old lineage, with large population size, but they do not have the expected high level of polymorphism.

Population size

It is generally agreed that populations containing very few individuals, or which have recently passed through a severe bottle-neck, lose variation through the random process of genetic drift. These effects have been reported in a variety of organisms - for example lizards (Webster *et al.*, 1972; Gorman *et al.*, 1975), rodents (Selander *et al.*, 1971, 1974), insects (Prakash, 1972; Saura *et al.*, 1973) and seals (Bonnell and Selander, 1974).

When this argument is extended to a general correlation between populations size and heterozygosity, it becomes much less convincing, since population sizes can rarely be measured accurately, especially when they are large. Soulé (1976) presents evidence that such a correlation exists, with estimates of populations size based on little more than guesswork. The correlation he observes is aided considerably by

a series of populations with $N_e \leqslant 10^4$ and $\bar{H} \leqslant 0.07$. These results all come from vertebrates, which may have low levels of variation for reasons other than low population size, as discussed earlier.

Estimation of effective population size in Hymenoptera is influenced by the fact that males are haploid. Wilson (1963) and Brian (1965) present expressions which do not take this into account, and which reduce to N_e = 2x the number of colonies, if each contains one singly mated queen. Wright (1969) gives the correct expression for effective population size at a sex linked locus as

$$N_e = \frac{9\, N_m N_f}{4N_m + 2N_f}$$

where N_m and N_f are the numbers of males and females respectively. If $N_m = N_f = N$ this reduces to $N_e = 1.5N$, or in the case of social Hymenoptera, 1.5x the number of colonies. In this idealized situation, N_e for a species of Hymenoptera is 3/4 that for an equally abundant diploid species, which may be seen as indicating a lower level of heterozygosity. However, there are several reasons why this conclusion may be misleading. Many species of Hymenoptera have very biassed sex ratios (Hamilton, 1967), and under some circumstances this may cause N_e to actually be *larger* for a haplodiploid species than for a diploid one (Crozier, 1976b). Also, many species of Hymenoptera contain partially or completely sterile worker castes, as well as showing inbreeding and parthenogenesis.

Even if a simple relationship between N_e and $\bar{H}$ is accepted this does not necessarily account for low heterozygosity in Hymenoptera. To do this, it is necessary to demonstrate a lower average N_e in the Hymenoptera species surveyed, than in the other organisms considered in Table 9.3. The three species of meat ants described here all cover extensive areas of Australia, and often reach high densities, so they cannot be said to have low N_e's. Nor is it likely that they have all passed

through a recent population bottleneck.

All of the forces discussed here as having an influence on levels of genetic variation have been invoked as "explanations" of observed results with some success in some circumstances, and a number of them may have some relevance to meat ants. Other factors, such as meat ants' large populations size and broad ecological niche, appear to predict just the opposite of the observed low level of heterozygosity. This problem appears even more acute when all the 22 species in Table 9.2 are considered together. These species are sufficiently diverse in ecology, degree of social behaviour, longevity, population size, and many other parameters, that to seek a single ecological explanation for low heterozygosity in all of them is doomed to failure. This approach also overlooks the one fundamental feature which they do all have in common - that is, haplodiploidy.

It was shown in Section 9.1 that the theory of selection at sex-linked loci may suggest a lower level of heterozygosity for such loci than for autosomal ones, although the extent of this reduction cannot be accurately predicted. This argument, as well as the preceding ecological ones, assumes that the type of variation being discussed is selectively maintained. It has been suggested that this is not so, and that much of the variation detected by gel electrophoresis is selectively neutral (*e.g.*, Kimura, 1968).

Effect of haplodiploidy on neutral alleles

The effect of haplodiploidy on the behaviour of neutral genes has been examined by Lester and Selander (1977). In particular, they were concerned with Kimura's (1962) formula for the probability of fixation of a mutant gene. They found that a new mutant gene was 1/3 more likely to achieve fixation in a haplodiploid population than in a diploid

one containing the same number of individuals, and would take fewer generations to do so. Both of these effects would reduce the amount of polymorphism for neutral alleles in haplodiploids, although the differences they found can largely be ascribed to the reduction in N_e, rather than to haplodiploidy *per se*.

The formulas used in this analysis, like most of the neutral mutation theory, are based on a series of simplifying assumptions. In particular, no distinction is made between males and females, and this may have a substantial effect on the results. In haplodiploidy a gene is more likely to have different frequencies in males and females, sex limitation may make it neutral in one sex and not the other, and a gene may be recessive in females while haploidy allows its immediate expression in males. Until these features can be incorporated into the theory, it is difficult to see how it can be used as a basis for firm predictions.

Lester and Selander (1977) also suggest that haplodiploidy may have effects on the heterozygosity of neutral loci, which are mediated by its effects on linkage. Haploidy in males means that their genome acts as a single linkage group , and the sperm produced by an individual is genetically completely uniform. This restriction on recombination and segregation may effect the behaviour of loci, both neutral and otherwise, in a number of ways.

In particular, the tight linkage of the genome may contribute to a high level of linkage disequilibrium in arrhenotokous species, as is found in organisms which use other types of parthenogenetic reproduction (Nei, 1975). The disequilibrium may be produced by selective or epistatic interactions between loci, population bottlenecks, or population admixture (Charlesworth and Charlesworth, 1973; Zouros and Krimbas, 1973; Thomson, 1977), but whatever its cause, its rate of decay is likely to be very slow in a species where re-assortment of genes occurs in only one sex.

Bennett and Oertel (1965; Bennett, 1963) have shown that the approach to linkage equilibrium for two sex linked loci may take approximately 1.5 times as long as that for two equally distant autosomal loci.

When an advantageous gene appears and progressively replaces its existing allele, changes occur in the frequency of alleles at linked loci. The ultimate fixation of this favoured gene will cause a reduction in heterozygosity in the region of chromosome surrounding it, with the extent of this reduction depending on the distance from the selected locus (Maynard Smith and Haigh, 1974). The importance of this phenomenon in reducing average heterozygosity will depend on the population size and the selective advantage of the new allele (Ohta and Kimura, 1975; Haigh and Maynard Smith, 1976), but there seems little doubt that reduced recombination will enhance its effect. Also, the rate of allelic substitution is accelerated by haplodiploidy, so this type of transient polymorphism (neutral or otherwise) will exist for a shorter time.

Another phenomenon influenced by linkage is so-called "associative overdominance" (Frydenberg, 1963). This refers to apparent overdominance at an unselected locus, caused by linkage disequilibrium with a nearby, genuinely heterotic locus. A few heterotic loci scattered through the genome may be able to retard the fixation of alleles at other loci, especially in small populations (*e.g.*, Ohta and Kimura, 1970, 1971; Thomson, 1977). Thus associative overdominance may contribute to the maintenance of heterozygosity, and to the evenness of gene frequencies across a species' range (Ohta, 1973). The opportunity for this process to act in Hymenoptera is restricted, both by the fact that heterosis can only manifest itself in females, and because selectively maintained polymorphisms may occur less often than in diploid species (Section 9.1). Thus associative overdominance may make a smaller contribution to heterozygosity in haplodiploid species than in others (Lester and Selander,

1977). Nevertheless, the combination of linkage disequilibrium and associative overdominance in experimental *Drosophila* populations can produce complex changes in the frequencies of sex linked genes, and lead to the short or long-term persistence of polymorphisms (Hedrick, 1976; Barker, 1977). Therefore the action of these forces in Hymenoptera can not be ruled out completely, although they have yet to be directly demonstrated.

Overview

Metcalf *et al.* (1975) interpreted low levels of heterozygosity in Hymenoptera to mean that much of the enzyme variation observed in natural populations is "selectively not neutral". However, the foregoing discussion suggests that haplodiploids should have reduced heterozygosity at loci which are selectively neutral as well as those which are not.

The "explanation" for a low level of heterozygosity in an individual species of Hymenoptera is probably best sought in a combination of several forces. The effects of haplodiploidy on neutral or nearly neutral loci must be considered, as well as the reduced probability of selectively balanced polymorphism. The universal nature of the reduction in heterozygosity in these species argues strongly that these general factors are of considerable importance. However, for individual species, they must be viewed in the light of other forces, such as environmental heterogeneity, inbreeding, and population size, if an understanding of the results is to be approached.

CHAPTER 10

GENETIC DISTANCES AND EVOLUTIONARY RELATIONSHIPS

10.1. Multilocus studies of isozyme data

In Chapters 4-6 I used the differences in allele frequency at particular loci as evidence of reproductive isolation between colour forms of meat ants. However, conclusions regarding reproductive isolation and evolutionary relationships are most reliable when they are based on evidence from the largest possible proportion of the genome. Therefore, the use of isozymes in systematics commonly involves the collection of gene frequency data from 20-25 loci in a series of natural populations (reviewed by Avise, 1974). These results may then be summarized in terms of a single measure of genetic distance (or similarity) between each pair of populations.

Measures of genetic distance and similarity have been calculated in a variety of different ways by different authors (*e.g.*, Cavalli-Sforza and Edwards, 1967; Balakrishnan and Sanghvi, 1968; Hedrick, 1971; Nei, 1971, 1972; Rogers, 1972). Several of these measures have been found to be highly correlated with each other, especially when very closely related taxa are considered (*e.g.*, Rogers, 1972; Avise, 1974; Hedrick, 1975). The major conclusions derived from examination of genetic distances are therefore unlikely to be seriously effected by a change from one distance measure to another.

Whichever distance measure is used, the data employed is ultimately derived from the electrophoretic mobility of proteins. This data is therefore subject to a number of uncertainties, introduced by convergent evolution, undetectable gene substitutions, and difficulties in the scoring of gels (Avise, 1974). Nevertheless, genetic distance measures provide a very valuable means of summarizing gene frequency data, whether derived from electrophoresis or some other technique. The next section deals with the application of one such distance measure to isozyme data in meat ants.

10.2. Genetic distances between meat ant populations

Table 10.1 shows the frequencies of alleles at 15 loci in nine populations of meat ants, including five colour forms. Some of this data has previously been presented in Chapters 4,5,6 and 9, but a complete summary is tabulated here for the first time. The gene frequencies are shown in the Table to two decimal places, but four were used in the calculation of genetic distances.

The distance measure used was D, of Nei (1971, 1972), since this is the measure which is most commonly used, and for which the largest amount of comparative data has been assembled, from a wide variety of organisms. If x_i and y_i are the frequency of allele i in populations X and Y respectively, then the identity of the two populations at a particular locus is given by

$$I = j_{XY}/\sqrt{j_X j_Y} \qquad \ldots\ldots\ldots\ldots (1)$$

in which $j_{XY} = \Sigma x_i y_i$; $j_X = \Sigma x_i^2$; $j_Y = \Sigma y_i^2$.

When a number of loci, both polymorphic and monomorphic, are considered together, the mean values of j_{XY}, j_X, and j_Y are used. The distance between the populations is then

$$D = -\log_e I.$$

The value of D, and its standard error, for each pair of populations, is shown in Table 10.2. The smallest distance found between any pair of populations is that between the Eyre Peninsula yellow form and Morgan black form. The distance between these is less than 0.001. The largest distance is 0.131, between the Eyre Peninsula black form and the Morgan blue form populations.

The standard errors of the genetic distances shown in Table 10.2 often approach the values of the distances themselves, and in several cases, are marginally greater. Contributing to these relatively large standard errors, are the fact that the distances are very small, and that

Table 10.1. Frequencies of 21 alleles at 15 loci in nine populations of meat ants. M = Morgan, EP = Eyre Peninsula, AR = Arthurton; P = red form, V = black form, B = blue form, SP = small purple form, Y = yellow form; N = average number of genes sampled per locus in each population.

	M-P	M-V	M-B	EP-P	EP-V	EP-B	EP-SP	EP-Y	AR-P
Gdh^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Me^{a}	1.00	1.00	1.00	1.00	0.00	1.00	0.25	1.00	1.00
Me^{b}	0.00	0.00	0.00	0.00	1.00	0.00	0.75	0.00	0.00
Mdh-1^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-2^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lap^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ldh^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Es-1^{o}	0.83	0.92	1.00	0.97	1.00	1.00	1.00	1.00	0.63
Es-1^{+}	0.17	0.08	0.00	0.03	0.00	0.00	0.00	0.00	0.37
Es-2^{o}	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
Es-2^{+}	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00
Es-3^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Amy^{a}	0.23	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
Amy^{b}	0.00	0.00	0.51	0.00	0.00	0.28	0.07	0.00	0.00
Amy^{c}	0.77	1.00	0.00	0.86	0.94	0.00	0.00	1.00	0.98
Amy^{d}	0.00	0.00	0.49	0.00	0.06	0.72	0.93	0.00	0.02
P-1^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P-2^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
G6pd^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ao^{a}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
N	48	45	51	19	15	16	14	15	57

Table 10.2. Genetic distances (Nei) between meat ant populations (above diagonal) and their standard errors (below diagonal. Abbreviations as in Table 10.1.

	M-P	M-V	M-B	EP-P	EP-V	EP-B	EP-SP	EP-Y	AR-P
M-P	-	.004	.051	.002	.076	.046	.098	.005	.007
M-V	.004	-	.061	.002	.070	.056	.108	.000	.006
M-B	.044	.056	-	.052	.131	.011	.063	.060	.070
EP-P	.001	.001	.047	-	.071	.047	.099	.001	.010
EP-V	.073	.072	.092	.072	-	.123	.063	.069	.081
EP-B	.046	.058	.008	.049	.092	-	.043	.055	.064
EP-SP	.072	.081	.045	.074	.061	.041	-	.107	.117
EP-Y	.004	.000	.055	.001	.072	.058	.081	-	.009
AR-P	.005	.006	.056	.009	.074	.058	.081	.010	-

only four loci (*Me, Es-1, Es-2,* and *Amy*) contribute to the variation in distance between pairs of populations. Inferences drawn from the resulting distances are therefore to be made with caution. Nevertheless, these distances provide the best summary of the genetic relationships between meat ant populations available at the moment.

The 36 possible pairwise comparisons among nine populations of meat ants, fall into three groups. Five measures of genetic distance between different populations within a colour form, lie in the range 0.002 to 0.070, with a mean of 0.020. Thirteen comparisons of sympatric populations of different colour forms, yield genetic distances in the range 0.001 to 0.123, with a mean of 0.061, and 18 comparisons of allopatric colour forms, are in the range 0.000 to 0.131, with a mean of 0.058.

The value of 0.02 for the genetic distance between geographic populations within a colour form, is very similar to that found within a species in a variety of other organisms (reviewed by Nei, 1975; Ayala, 1975). However, the average distance of approximately 0.06, between populations of different colour forms, is much lower than that normally found for sibling species, or even for subspecies. For example, in the *Drosophila willistoni* group, the average genetic distance between subspecies is 0.230, and that between sibling species is 0.581 (Ayala, 1975). Therefore, it appears that the very close *morphological* similarity between the colour forms of meat ants is accompanied by close *genetic* similarity, despite the fact that some forms have quite different alleles or allele frequencies at some loci. Some of the forces which may be responsible for this lack of divergence between colour forms are considered in Chapter 13.

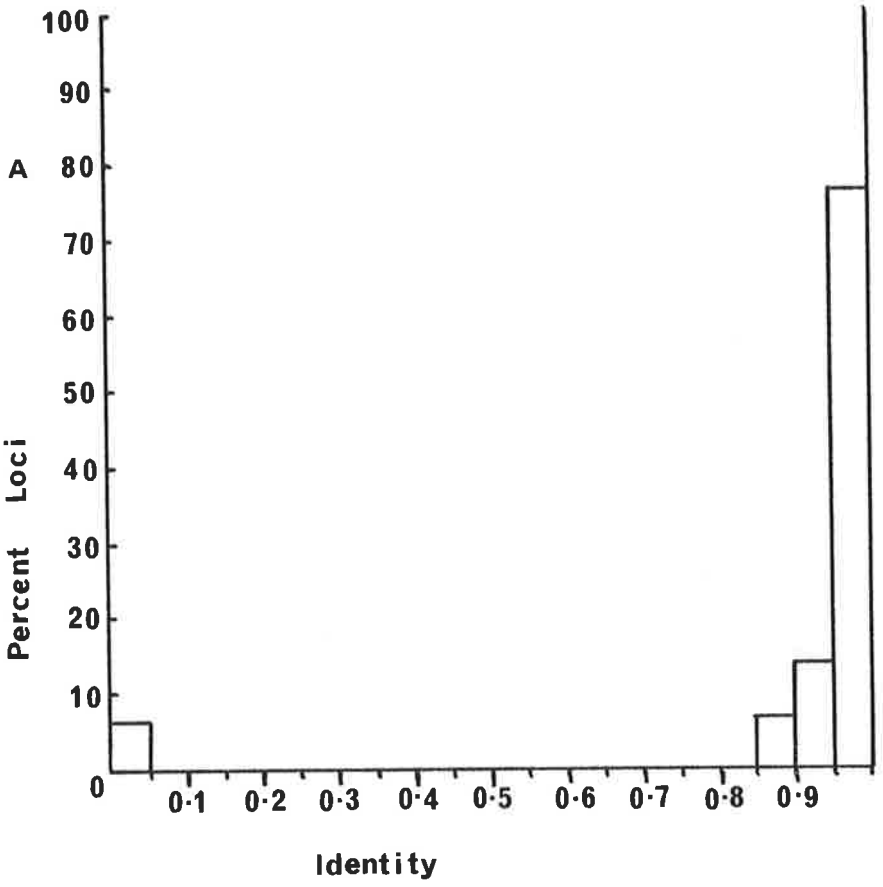
The genetic distances discussed so far have been derived by considering all loci simultaneously. The genetic relationships between populations may also be examined by considering each locus separately.

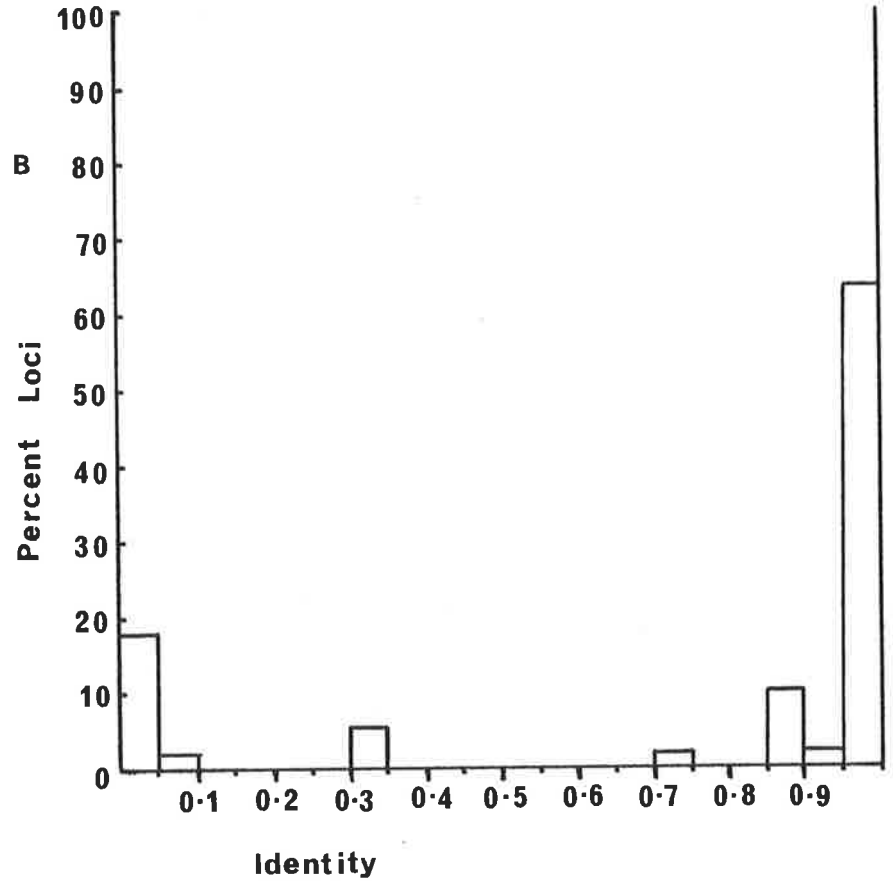
This is usually done in terms of genetic similarity, or identity, rather than distance, with the single locus identity between two populations calculated from Equation (1) above. The results of this analysis are presented in Figure 10.1, considering both comparisons of geographic populations within colour forms (A), and comparisons of populations of different colour forms (B).

In these analyses, I have not included the 11 loci which are identical and monomorphic in all nine populations. Nevertheless, both analyses still reveal a large proportion of cases in which populations have very similar or identical allele frequencies for the other four loci. In the case of geographic populations within colour forms, the only exception is for *Malic enzyme* in the black form, where two populations appear to be fixed for different alleles. However, when populations of different colour forms are compared, there is a substantial rise in the frequency of loci with similarity values at or near zero. The resulting U-shaped frequency distribution (Figure 10.1B) is characteristic of comparisons of populations belonging to different sibling species (Avise, 1974; Ayala, 1975). This is, of course, just another way of looking at the reproductive isolation between colour forms. The cases where there is little or no similarity between forms are almost entirely due to the existence of different *Amylase* alleles in different forms, as discussed previously.

Figure 10.1A. The distribution of single-locus genetic identity between populations of meat ants considering only geographic populations within colour forms (16 comparisons).

Figure 10.1B. The distribution of single-locus genetic identity between meat ant populations, considering only populations of different colour forms (128 comparisons).


A
Percent Loci
100
90
80
70
60
50
40
30
20
10
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
Identity


B
Percent Loci
100
90
80
70
60
50
40
30
20
10
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
Identity

10.3. Evolutionary interpretation

Once a matrix of genetic distances such as that in Table 10.2 is constructed, inferences may be drawn from it concerning the phenetic and phylogenetic relationships among the populations involved. The first step in this process is the construction of a dendrogram summarizing the results.

Many different techniques are available for the construction of dendrograms from distance matrices (reviewed by Sneath and Sokal, 1973), most of which can be applied to genetic distances based on isozyme data. These techniques all rely on the clustering of populations in such a way that the most similar populations are combined into groups. These groups are then combined with other groups, to which they have progressively diminishing degrees of resemblance. Whatever the clustering method used, the resulting dendrograms are of particular value when the results can be compared with relationships derived from other types of evidence.

For example, an extensive study of the *Drosophila willistoni* group (reviewed by Ayala, 1975), reveals taxa at a number of stages in the process of evolutionary divergence, including geographic populations, subspecies, semispecies and sibling species. A dendrogram constructed from allele frequencies at 36 isozyme loci in 14 taxa (Figure 8 of Ayala, 1975), reveals relationships which are extremely similar to those indicated by data from chromosomal polymorphism, morphology, and reproductive isolation. Indeed, it is commonly found that there is good agreement between the relationships indicated by genetic distances, and those based on more conventional evidence.

However, exceptions to this rule can easily be found. Johnson and Selander (1971) found a general agreement between relationships based on electrophoretic and morphological data in Kangaroo Rats (*Dipodomys*), but there were some interesting differences as well. One morphologically based classification placed *D. deserti* and *D. spectabilis* close together,

but isozyme data indicated that they were only distantly related. Avise and Selander (1972) found that cave and surface populations of the fish *Astyanax mexicanus* were morphologically quite different, but in terms of isozymes they were very similar. A further example concerns the pupfish (*Cyprinodon*) populations in Death Valley, California (Turner, 1974). Dendrograms constructed from morphological and isozyme data on five species are strikingly different, indicating that the morphological divergence of these species was not accompanied by extensive changes in the frequency of alleles at the isozyme loci which were sampled.

Figure 10.2 is a dendrogram constructed from the genetic distances between the meat ant populations shown in Table 10.2. The clustering method used to derive this dendrogram was the Unweighted Pair-Group Method of Averages, or UPGMA (Sneath and Sokal, 1973, p. 230), which is one of the simplest methods available, and one of the most commonly used. This dendrogram indicates that the two blue form populations cluster together, and are then joined by the small purple form, as expected. The other major group of populations includes the red, yellow, and black forms. The co-phenetic correlation between the distances shown in the dendrogram and those in the original distance matrix, is 0.85, indicating that the construction of the dendrogram has not seriously distorted the relationships between the populations. Nevertheless, in the red-yellow-black group, the genetic relationships of the populations do not coincide with their similarities on the basis of colour. The three red form populations do not cluster together, and the two black form populations are the most distant from each other of any pair of populations in this group. The dendrogram shown therefore does not coincide with the presumed phylogenetic relationships between these nine populations.

It is well known that phenetically-based dendrograms such as the one I have constructed, do not accurately reflect phylogeny unless the rate of evolution is constant over all phyletic lines (*e.g.*, Sneath

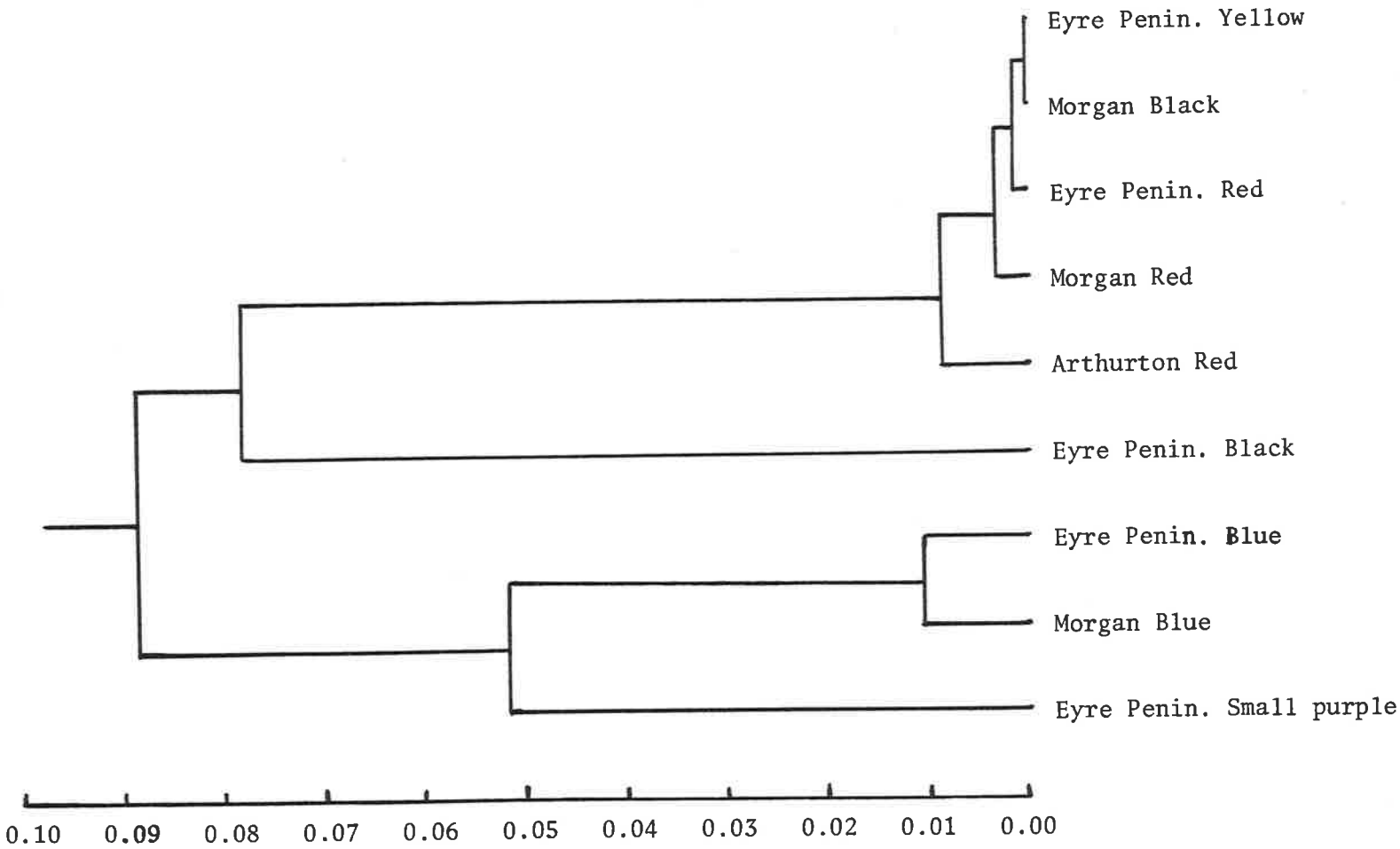


Figure **10·2**

Dendrogram summarizing Nei's genetic distances between 9 populations of Meat Ants.

and Sokal, 1973; Avise, 1974; Nei, 1975). The fact that the electrophoretic differences between populations do not always coincide with differences between colour forms, may therefore suggest that gene substitutions have taken place more rapidly in some lines than in others.

In Chapter 7 I suggested that stochastic processes had probably played a major part in producing the patterns of geographic variation in allele frequency found within colour forms. If so, the process of genetic drift appears to have taken place at different rates in different populations. In particular, the large genetic distance between the two black form populations has been caused by their fixation for different alleles at the *Malic enzyme* locus, while populations within other forms have not diverged to the same extent.

Genetic distance values have been used in some cases as measures of the time since the separation of two populations or species (*e.g.*, Yang *et al.*, 1974; Zimmerman and Netjek, 1977). These times can then be related to climatic or other factors which may have been associated with the separation of the populations, such as colonization events or glacial periods. However, the fact that rates of evolution appear to have been much faster in some lines than others, means that the genetic distances between meat ant populations are not related in a simple way to evolutionary time.

Furthermore, it is possible that the differential divergence of populations in different colour forms has been brought about by selection rather than by random genetic drift. However, the demonstration of the action of selection on isozyme polymorphisms, requires the large scale collection of data on environmental variables, and on the biochemical properties of the different allelic products. It is unlikely that this will be possible for meat ants in the near future. Also, the conclusions drawn from genetic distances would be strengthened if their standard errors could be reduced. This would involve collection of data from a

substantially increased number of polymorphic loci, which are likely to be difficult to identify.

Nevertheless, it appears from the present data that the evolutionary processes operating in meat ants have resulted in considerable genetic differentiation of populations within colour forms. This differentiation within forms is sometimes of the same order of magnitude as the differences between forms, perhaps because the various forms are very similar, both genetically and morphologically.

CHAPTER 11

KARYOTYPE STUDIES

The chromosomes of meat ants were first described by Crozier (1968a). He found that the karyotype consisted of a diploid number of 18, with six pairs of large metacentric or sub-metacentric chromosomes, and three pairs of small chromosomes. In the blue form, these three small chromosomes all had clearly visible short arms. In the red form, only one of the pairs of small chromosomes was submetacentric, while the other two were acrocentric, with negligible short arms. With the small amount of evidence available at that time, it was not possible to decide whether this difference in the morphology of the small chromosomes indicated a difference between species, or cytological variation within a single species.

Since that time, many more Australian ant karyotypes have been described. Imai, Crozier and Taylor (1976, 1977) review this information, and use it as the basis of a general model of ant chromosome evolution, in which Robertsonian changes, pericentric inversions, and "growth" of constitutive heterochromatin, play a major part. In the case of Robertsonian changes, Imai *et al.*, (1976, 1977) argue that centric fission has occurred more frequently than fusion, and that the general evolutionary trend in ant chromosome numbers has been upward. This general model provides a background for the detailed examination of karyotypic changes in specific groups, especially in the case of groups of closely related species.

I have examined the chromosomes of seven of the eight currently recognized colour forms of meat ants, most of which have not previously been examined cytologically. The location of the nests from which animals were collected, the number of specimens examined, and the number

of reliably scorable divisions examined in each, are shown in Table 11.1. These sample sizes are very low, and the collections come from only a restricted part of the range of each colour form. Any conclusions drawn from the results must therefore be regarded as provisional. Nevertheless, the karyotypes I have found are consistent with the hypothesis that Robertsonian fission, pericentric inversion, and the tandem growth of constitutive heterochromatin, have all played a part in the chromosomal evolution of these colour forms.

The chromosome number in all cases is 2n=18 (Figures 11.1, 11.2.). Imai *et al.* (1977) placed meat ants in a group with other 2n=18 species of Dolichoderinae, and suggested that this group was related to 2n=16 species by a single Robertsonian change. Chromosome 2 of the 2n=16 species appears to correspond to chromosomes 7 and 8 in the 2n=18 species, despite the fact that the latter have obvious short arms. In the case of chromosome 8, these usually take the form of recognizable "satellites" in all the colour forms of meat ants I have examined (Figures 11.1 and 11.2). The size of the short arms on chromosome 7 appears to be variable - minimal in the red form (Figure 11.2 B and C), noticeable in the small purple and yellow forms, and slightly larger in the black form (Figure 11.1).

Imai *et al.* (1977) also report one colony of the blue form containing a pericentric inversion in chromosome 7, making it appear sub-metacentric, instead of acrocentric as it is in other 2n=18 species. The two nests of the blue form I have examined both contained individuals in which chromosome 7 was acrocentric (*e.g.* Figure 11.2 A).

However, chromosome 7 appears to be variable in *sanguineus*. It was acrocentric in two specimens from nest 448, but in nest 447, one individual out of the five examined appears to be heterozygous for a pericentric inversion (marked with a dot in Figure 11.2 D). The other four individuals from this nest had the normal acrocentric chromosome 7

Table 11.1. Location of nests from which karyotypic information has been obtained, and the number of individuals and cells examined for each. All individuals were worker pre-pupae unless other wise indicated.

	Nest number	Location	Individuals examined	Cells scored
Blue form	126	Whyalla S.A.	1	3
	445	Whyalla S.A.	2	6,5
Red form	134	Kimba S.A.	2	1,1
	408	Blackwood S.A.	1 + 1♂	4,5
	425	Truro S.A.	1 + 1♂	2,2
	440	Kimba S.A.	1	3
	460	Eudunda S.A.	1♂	4
Small purple form	416	Mundoora S.A.	2	1,1
	431	Whyalla S.A.	1	2
	444	Kimba S.A.	2	2
Yellow form	136	Kimba S.A.	2♂	4,1
	433	Kimba S.A.	1	3
	435	Kimba S.A.	1♂	3
Black form	333	Whyalla S.A.	2	3,3
	429	Whyalla S.A.	1	1
	446	Whyalla S.A.	2	2,4
sanguineus	447	Townsville Qld.	5	6,1,3,4,5
	448	Townsville Qld.	2	6,5
Dark Yellow form	450	Springsure Qld.	1♂	8
	451	Springsure Qld.	1	4
	453	Emerald Qld.	2	1,3

Figure 11.1. Karyotypes of meat ants

A Blue form worker - nest 445

B Small Purple form worker - nest 431

C Black form worker - nest 429

D Yellow form worker - nest 433

E Yellow form male - nest 136

Bar scale represents 10 microns.

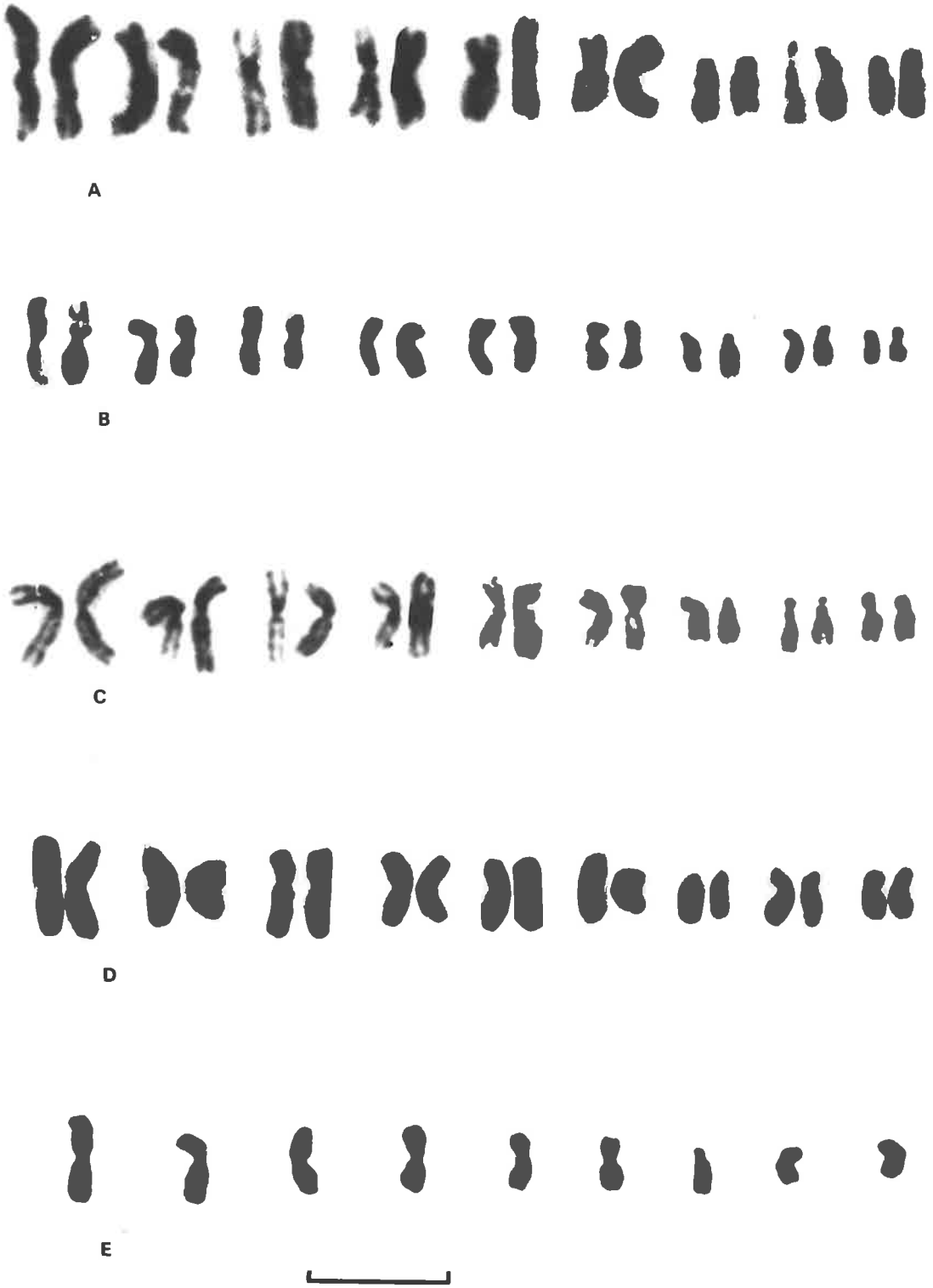

A
B
C
D
E

Figure 11.2. Karyotypes of meat ants

A Dark Yellow form worker - nest 453

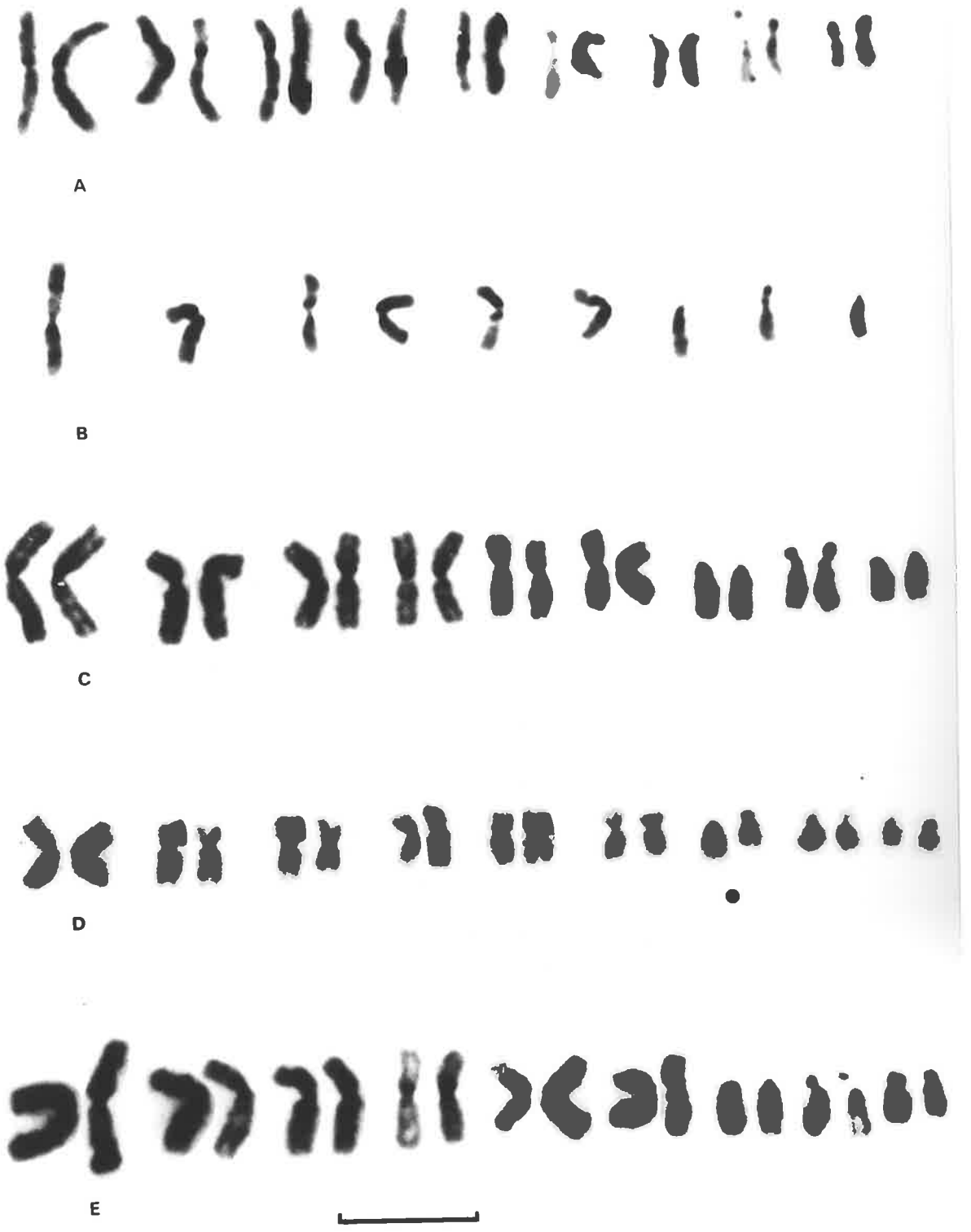
B Red form male - nest 425

C Red form worker - nest 440

D *sanguineus* worker - nest 447
Dot indicates possible heterozygosity for a pericentric inversion in chromosome 7.

E *sanguineus* worker - nest 447
This is a different individual from that shown in D, but from the same nest.

Bar scale represents 10 microns.


A
B
C
D
E

(*e.g.* Figure 11.2 E). If this is a genuine inversion, it may have arisen independantly of that in the blue form, although with the present sample sizes, its existence in other colour forms cannot be ruled out.

Chromosome 9 also shows morphological variation. It appears to have extremely small short arms or none at all in the red form (Figure 11.2 B and C) as reported by Crozier (1968a). In other colour forms, this chromosome has short arms of varying length, which may have been produced by the tandem growth of constitutive heterochromatin. These arms are short in *sanguineus* (Figures 11.2 D and E), but quite conspicuous in the blue, small purple, black, yellow, and dark yellow forms (Figures 11.1, 11.2).

These tentative findings require verification on the basis of larger collections, from widely separated localities within the range of each colour form. A large scale survey should also include C-banding analysis, to aid in the identification of the three pairs of small chromosomes, as distinguishing between these is sometimes difficult.

Until the extent of chromosomal variation *within* colour forms is more carefully examined, it is not possible to use karyotypes to distinguish *between* forms. The only possible exception is that chromosome 9 is acrocentric in all colonies of the red form so far examined (Crozier, 1968b; and present results), while this chromosome appears to have definite short arms in all other forms. The difference between the karyotypes of the red and blue forms reported by Crozier (1968a) may be a special case of the general difference between the red form and the other six forms examined here. This difference is consistent with the reproductive isolation of the red and blue forms suggested by allozyme data.

To summarize, the speciation processes which have occurred in the meat ant group do not appear to have been accompanied by conspicuous chromosomal re-arrangements, although there are slight differences between the karyotypes of some colour forms, as well as some variation within forms.

CHAPTER 12

MORPHOLOGICAL VARIATION

In Chapter 2, I pointed out that it is not possible to distinguish between the colour forms of meat ants on the basis of clear cut structural differences. However, it may be possible to recognize quantitative differences between them if large samples are considered. This Chapter attempts to examine the extent of morphological variation, both within and between colour forms.

12.1. Overall comparison of colour forms

Many techniques are available for the simultaneous analysis of several or many morphological variables (reviewed by Sneath and Sokal, 1973; Clifford and Stephenson, 1975). One such technique is canonical variate analysis, which in practise, is equivalent to multiple discriminant analysis. This technique is especially useful where there is some *a priori* reason for sorting specimens into groups. Several variables are then measured for each specimen, and this raw data is transformed into new quantities called canonical variates. These variates are calculated so as to maximize the differences between groups, while minimizing the variation within groups. The canonical variates therefore discriminate between groups more sharply than any of the original variables.

Delany and Healy (1964) measured ten variables in island populations of the field mouse *Apodemus sylvaticus*, and found that most of the morphological differences between populations on different islands could be summarized into two canonical variates. The populations did not fall into distinct clusters which could be recognized as different species, and some groups which had been referred to as subspecies, were shown by this analysis not to be morphologically distinct.

Rostron (1972) applied canonical analysis to morphological variation in the skulls of gazelles (*Gazella*), and found that the five recognized species and subspecies were indeed morphologically distinct. Furthermore, this analysis allowed the recognition of some specimens which could not be identified on the basis of the previously existing classification system.

In the Hymenoptera, DuPraw (1964, 1965) used multiple discriminant analysis of wing measurements of honeybees (*Apis* spp.) as the basis of his "non-Linnean taxonomy". His work was partly motivated by the existence of several conflicting classifications of these organisms, conflicts which he suggested could be eliminated if a purely numerical method of classification was used.

In meat ants, Greenslade (unpublished) has measured workers from seven populations, including three populations of the red form, and one each of the blue, small purple, yellow, and black forms. Each sample consisted of 40 specimens, with no more than three from any one nest. Three variables were measured - "head width at the widest point; head length in the midline from the vertex to the apex of the clypeus; length of trunk from the anterior margin of the pronotum to the posterior margin of the propodeum". The means and standard deviations of these variables do not allow clear distinctions to be drawn between the different populations (Table 12.1). However, with the assistance of Dr. R. J. White* I have performed a canonical discriminant analysis of these data, to determine whether this procedure could reveal relationships which were not previously apparent.

Figure 12.1 summarizes the result. The points plotted are the centroids (multivariate means) of each population, and their 95%

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*Present address, Dept. of Biology, University of Southampton, England.

Table 12.1. Mean and standard deviation (millimetres) of three variables in seven populations of meat ants. Each figure is based on 40 ants, with no more than three used from any one nest. Data from Greenslade (unpublished).

Locality	Form	Abbreviation	Trunk length	Head length	Head width
Mt Lofty Ranges	Red	MLP	3.13±0.14	2.01±0.09	1.65±0.11
Flinders Ranges	Red	FP	3.06±0.24	1.92±0.15	1.90±0.18
Eyre Peninsula	Red	EPP	3.22±0.24	2.08±0.10	2.01±0.14
Eyre Peninsula	Yellow	EPY	3.21±0.20	2.08±0.09	1.99±0.11
Eyre Peninsula	Small Purple	EPSP	2.93±0.22	1.92±0.13	1.79±0.14
Eyre Peninsula	Black	EPV	3.18±0.26	2.06±0.14	2.01±0.18
Eyre Peninsula	Blue	EPB	2.61±0.15	1.92±0.09	1.82±0.10

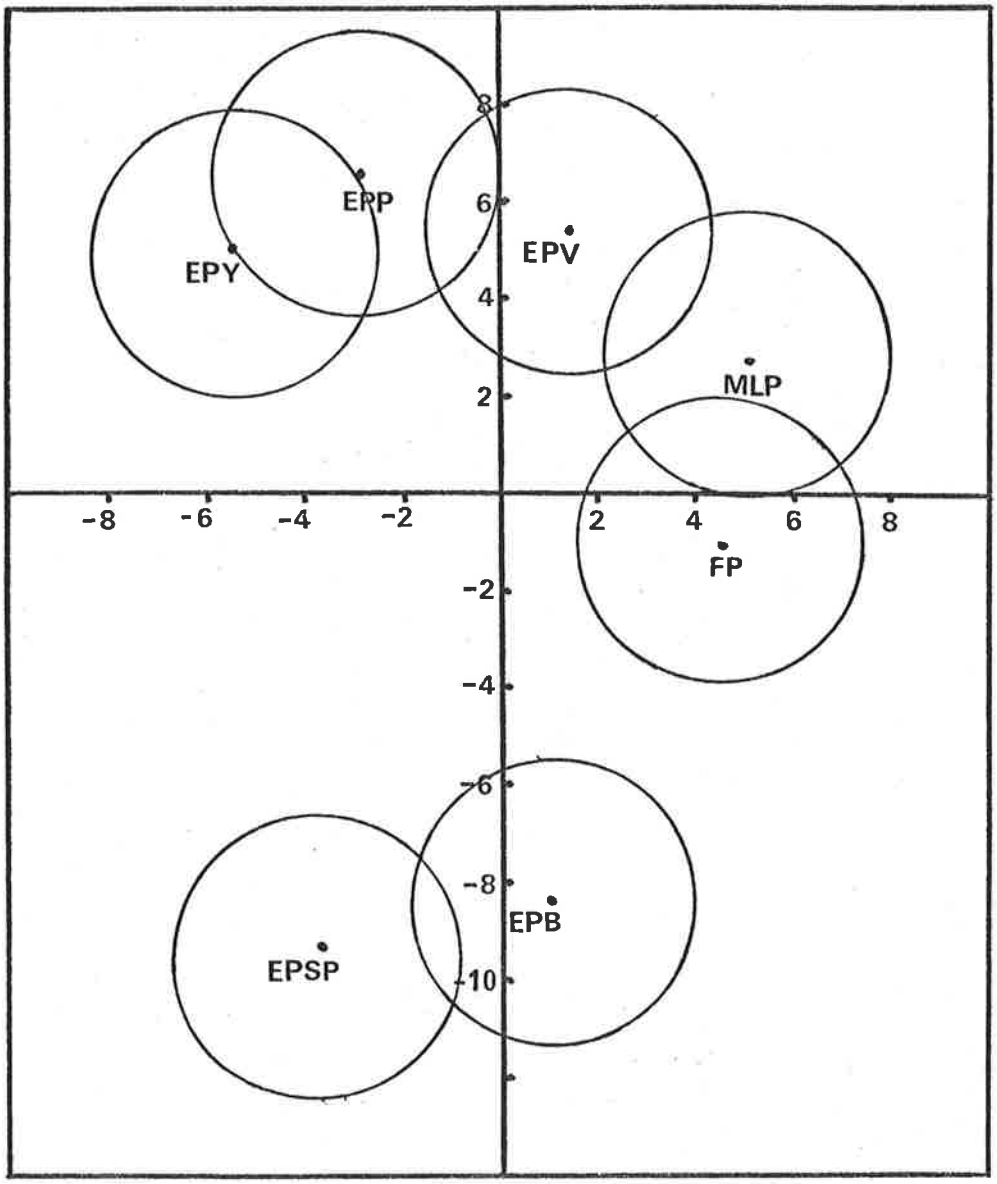


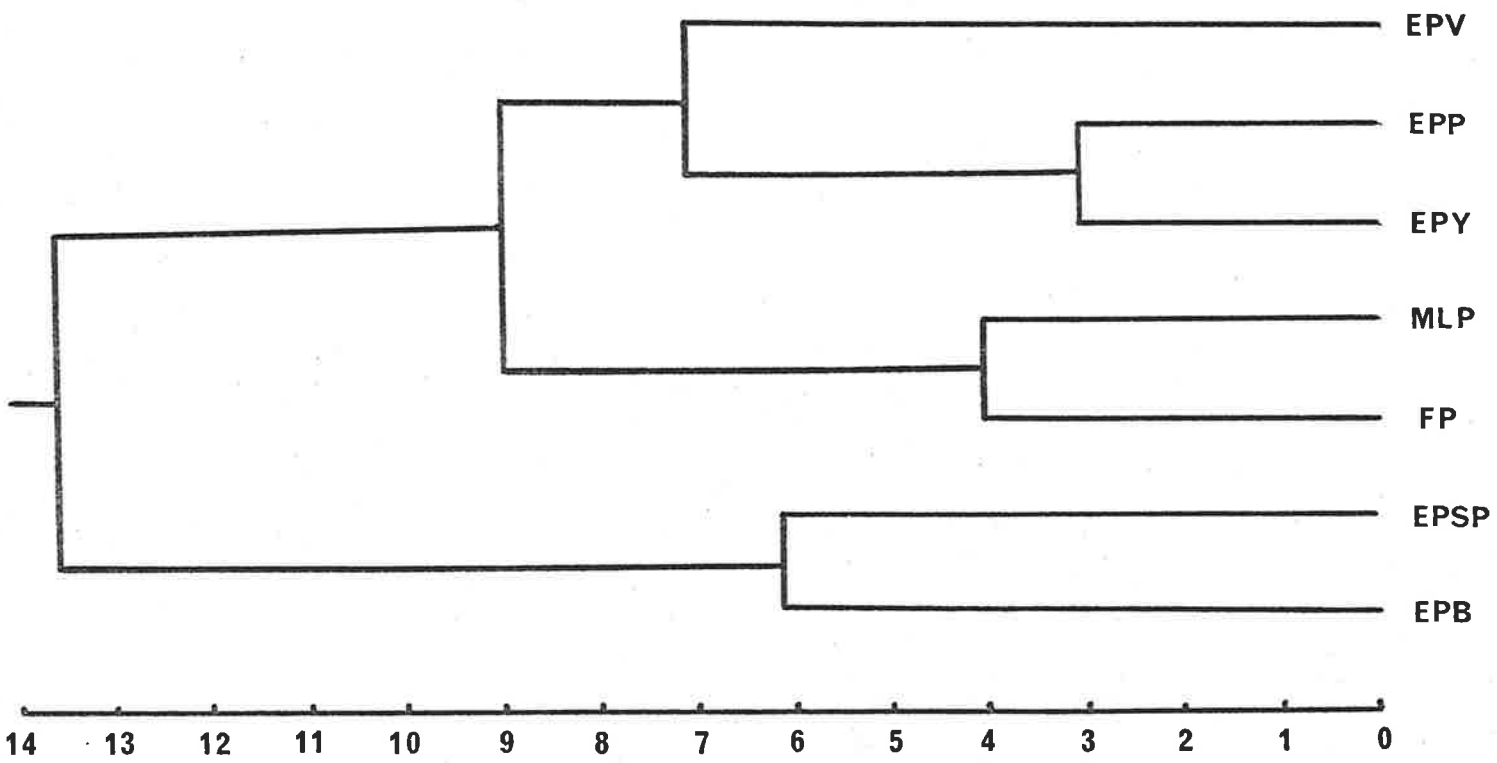
Figure 12.1

Canonical discriminant analysis of seven populations of meat ants. Points plotted are group centroids with their 95% confidence limits.
Vertical - First canonical axis x10
Horizontal - Second canonical axis x10
Populations :-
EPY = Yellow form, Eyre Peninsula
EPP = Red form, Eyre Peninsula
EPV = Black form, Eyre Peninsula
MLP = Red form, Mount Lofty Ranges
FP = Red form, central Flinders Ranges
EPB = Blue form, Eyre Peninsula
EPSP = Small Purple form, Eyre Peninsula

confidence limits. The first canonical axis is the direction in which there is maximum discrimination between groups. This axis accounts for 68.34% of the between-group variation, and values along this axis are highly correlated (r = 0.94) with trunk length (*i.e.* overall body size). The second canonical axis accounts for a further 26.12% of the between-group variation, so only 5.54% of this variation is not represented in Figure 12.1.

The seven populations fall into two groups, one containing the blue and small purple forms, and the other containing the red, yellow, and black forms. As with other types of data, the major distinction is between forms which build mounds and those that do not, and once again there does not seem to be any simple subdivision of the populations in the mound-building group in a way which corresponds with the differences between colour forms.

To demonstrate this point more clearly, I have calculated the Euclidean distance between each pair of populations, using their co-ordinates on each of the three canonical axes. The resulting matrix of distances was then subjected to UPGMA cluster analysis, as with the genetic distances in Chapter 10. Figure 12.2 shows the resulting dendrogram. The co-phenetic correlation between the distances shown in the dendrogram, and those in the original distance matrix, is 0.87. The populations of the blue and small purple forms cluster together, but the three populations of the red form do not. These populations are not exactly the same as those in Chapter 10, but the result is very similar - there appears to be sufficient geographic variation in the red form in both morphology and gene frequencies, to obscure its relationships with other forms, on the basis of the data which is presently available.



Morphological distance x10

Figure 12.2

Dendrogram derived from canonical discriminant analysis of seven populations of meat ants. Populations and abbreviations as in Figure 12.1.

12.2. Temporal variation in four nests

There is some evidence to suggest that meat ant workers are subdivided into ground foragers and tree foragers (Chapter 2). Despite the fact that there are no distinct castes, each worker seems to perform only one of these functions. Many species of ants are known to have workers which perform different tasks at different ages (reviewed by Wilson, 1971). The tasks recognized include foraging, brood care, colony defense, and nest building. Several questions therefore arise - a) in meat ants, can tree and ground foragers be distinguished morphologically? b) does an individual worker carry out different tasks at different ages?, and c) if so, do the same changes take place simultaneously in different nests and different forms?

The data used to examine these questions comes from measurements made by P. J. M. Greenslade and C. Kirkby (unpublished). It consists of the same three variables as in the previous section, with workers subdivided into three putative castes. They are tree foragers, collected from trees near the nest, ground foragers, collected from the ground surface, and nest workers collected from within the nest. Four nests were used, including two red form nests 60 metres apart near Belair (10 km. southeast of Adelaide), and one nest each of the red and blue forms 400 metres apart near Cambrai (80 km. northeast of Adelaide). Collections were made from each nest (where possible) at approximately monthly intervals from July 1973 to May 1974.

It was sometimes difficult to obtain tree and ground foragers in unfavourable weather, so the set of data which is most complete is that for nest workers. The monthly collections of nest workers from each nest were pooled and subjected to a preliminary canonical analysis, the result of which is shown in Figure 12.3.

Values along the first canonical axis are once again highly

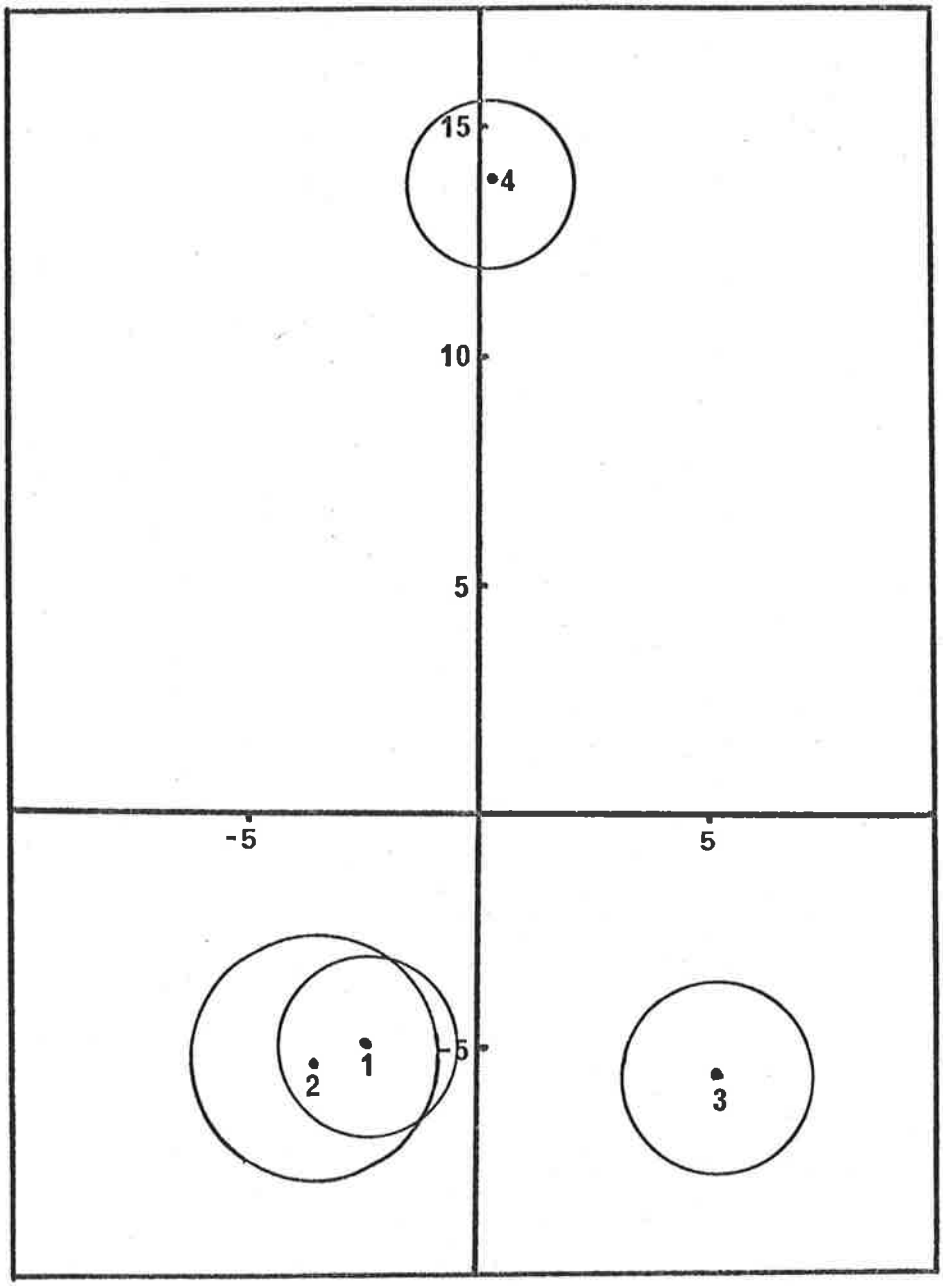


Figure 12.3.

Canonical discriminant analysis of nest workers from four nests. Points plotted are group centroids and their 95% confidence limits.
Vertical - First canonical axis x10
Horizontal - Second canonical axis x10
Nests:-
1 Red form Belair nest 1 (111 workers)
2 Red form Belair nest 2 (66 workers)
3 Red form Cambrai nest P5 (93 workers)
4 Blue form Cambrai nest V8 (105 workers)

correlated with trunk length (*i.e.*, overall size) (r = 0.99), so the Figure indicates a considerable size difference between the workers of the two colour forms. Workers from the two Belair nests are virtually indistinguishable on this basis, while the Cambrai red form collection has a very similar value to these on the first canonical axis, but is displaced somewhat along the second.

The three types of workers were then considered separately. It was found that the three groups from each nest plotted out extremely close together, with highly overlapping confidence limits, while the relative positions of collections from the four nests were retained. The differences between the three types of workers within a nest are therefore much smaller than the differences between nests, and there is no evidence of a consistent size difference between the three worker groups.

Monthly variation in all three types of workers was then examined for each nest. The most complete set of data is that from red form nest P5, at Cambrai. The value of the first canonical axis for each monthly collection from this nest is shown in Figure 12.4. Each point is based on nine workers, except for the first two collections of nest workers, in which only six were measured.

It is clear from this Figure that there is considerable temporal variation in the size of these workers, and that all three types of workers vary simultaneously. Data from the other three nests is less complete, but indicates the same trend. Since data was only collected over one year, it is not possible to determine whether these changes are part of an annual cycle. However, the data provide no evidence to suggest that the three worker types are consistently different in size, or that individual workers carry out various tasks at different ages. If the workers did behave in this way, size fluctuations in the three

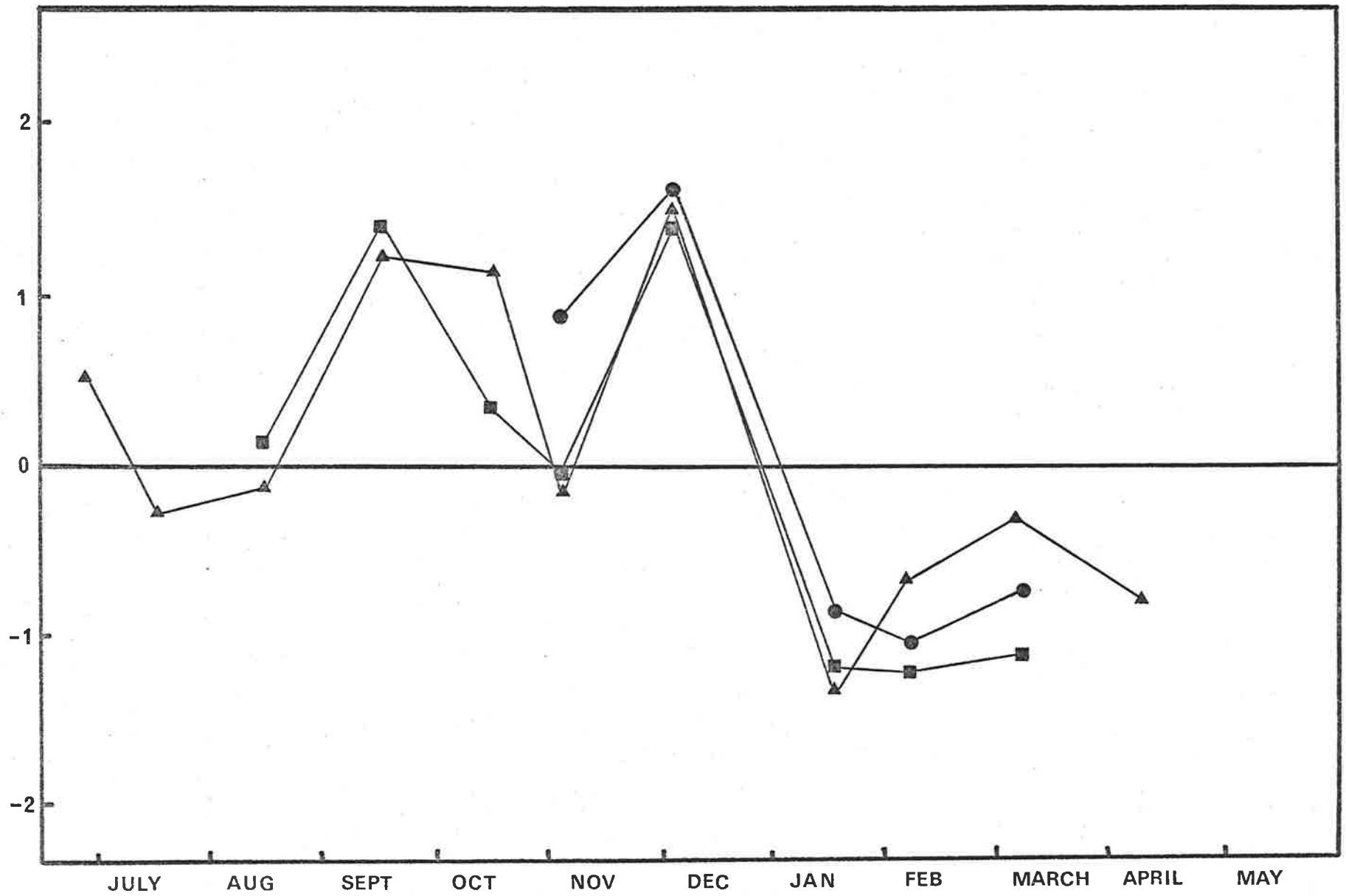
Figure 12.4.

Temporal variation in the size of workers from nest P5 (Cambrai, Red form).

Horizontal - time: July 1973 - May 1974

Vertical - First canonical axis

- ● Tree foragers
- ■ Ground foragers
- ▲ Nest workers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worker types might be expected to be out of phase with each other, and this does not appear to be the case in this body of data.

The three worker types were therefore pooled for an overall comparison of temporal variation in the four nests. Figure 12.5 shows the result. The mean number of ants contributing to each point in this case is 18.3. Workers from red form nests 1 and 2 at Belair show almost identical patterns of temporal variation. The red form ants from Cambrai show variation which is similar to that for the Belair nests, but with some differences, especially from December onwards. The large change in the size of ants in Cambrai nest P5 in December may be associated with a change in the number of entrance holes, from 12 on 15/10/73, to 25 on 5/11/73, and then 35 on 5/12/73. The number of holes in the other two red form nests remained within fairly narrow limits for the whole period of sampling.

The blue form nest at Cambrai contained workers which were quite distinct from those in any of the red form nests, at all times of year. It is therefore unlikely that a spurious difference between these forms could be created by sampling them at different stages in an annual cycle.

To summarize the results of this section - there is substantial morphological variation among the workers within nests, which is of approximately the same order as the difference between nests of the same colour form. The difference between the red and blue forms appears to be a consistent one. However, until a larger scale survey is undertaken, including other colour forms, it is not possible to decide whether the measurements and analysis used will yield a reliable method of identifying specimens.

Figure 12.5.

Temporal variation in the size of workers from four nests.

Horizontal - time: July 1973 - May 1974

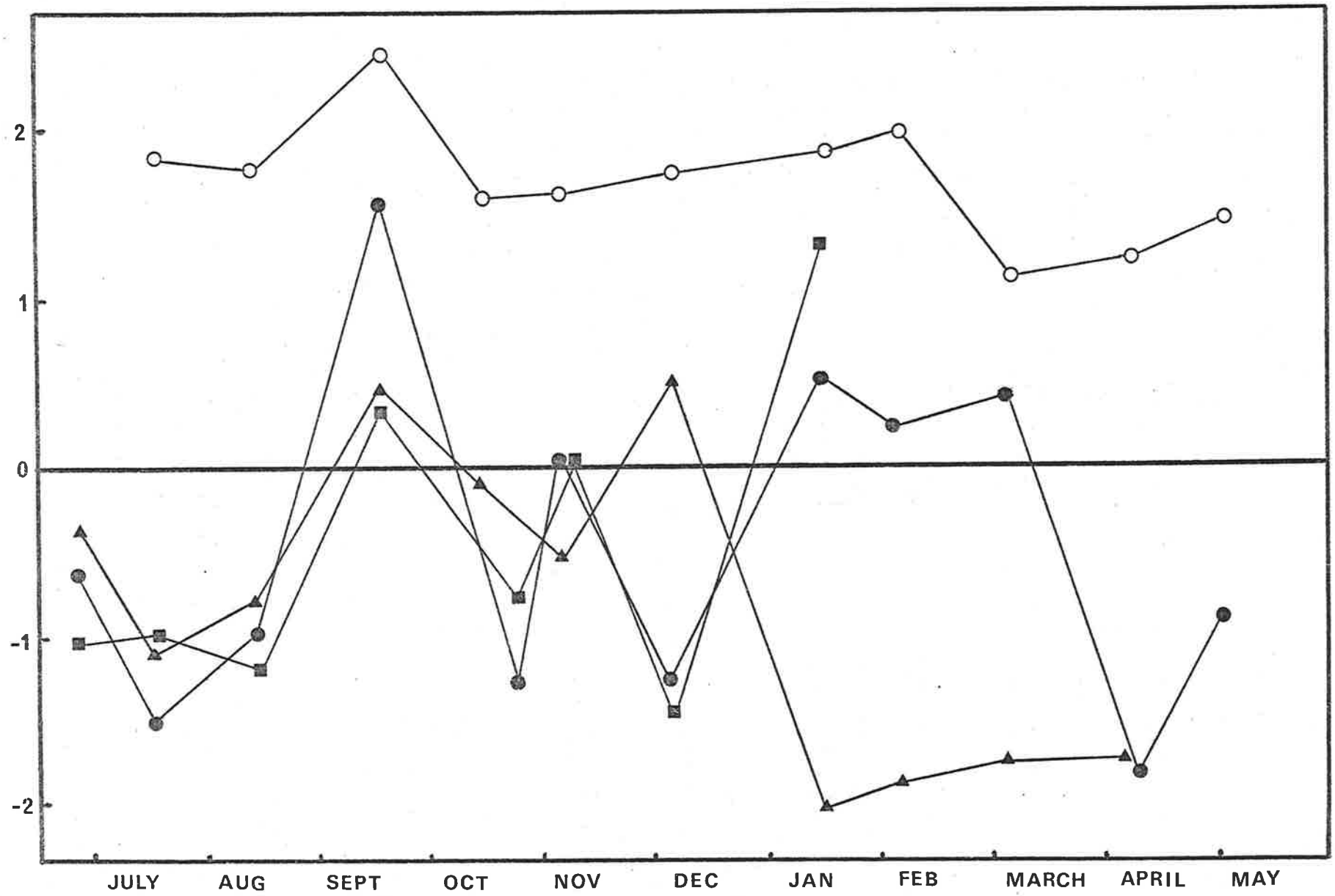
Vertical - first canonical axis

● Belair Nest 1 - Red form

■ Belair Nest 2 - Red form

▲ Cambrai Nest P5 - Red form

○ Cambrai Nest V8 - Blue form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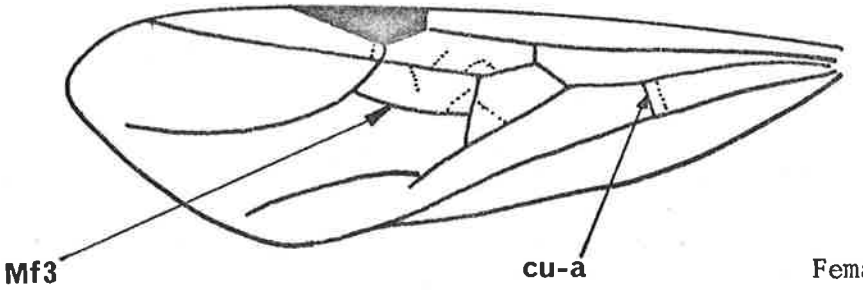
12.3. Variation in wing veins

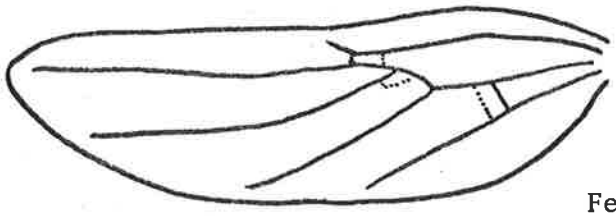
Most of the variation examined till now, whether allozymic, chromosomal, or morphological, has dealt mainly with workers. However, males and females may offer extra types of information which cannot be obtained from workers. This is especially true in the case of two types of structures which workers do not possess - wings and genitalia.

The pattern of veins in the wings is a character which is commonly used in insect taxonomy, and the Hymenoptera are no exception. However, Hymenoptera wing veins are quite different from those of many other orders, and several different systems of nomenclature have been derived to describe them (reviewed by Ross, 1936, 1948). In the case of ants, it is sometimes found that there is variation in the pattern of venation within a species (*e.g.*, Brown and Nutting, 1950; Delage-Darchen, 1973). Even within individuals, specimens are known with wings which would normally be classified as belonging to different genera (W. L. Brown Jr., personal communication). This instability in the venation pattern may be associated with the loss of a vein during the evolution of a lineage. Once such a structure is lost, it may not re-appear subsequently, except as an unstable "atavism" which is not fully developed (Brown and Nutting, 1950).

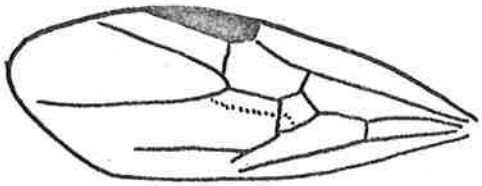
I have observed this instability in some of the wing veins of meat ants. Figure 12.6 illustrates some of the variants found. The most conspicuous variation involves the vein labelled Mf3 ("third free abscissa of the Median vein"). It is usual for this vein to be fully developed in females, and completely absent from males. In a minority of females, this vein was very thin or broken, and in some males it was present to varying degrees (as also noted by Emery, 1913). The other type of variation noted was in the presence of extra short veins, as shown in the Figure. In a small collection of the red form, one or more



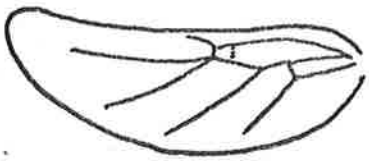
Female forewing



Female hindwing



Male forewing



Male hindwing

Figure 12.6.

Wing vein variation in meat ants.
Solid lines indicate the basic pattern of veins, and dotted lines indicate additional variable veins.
Mf3 = third free abscissa of the Median vein
cu-a = cubito-anal crossvein

of the additional veins shown were present in approximately 30% of the females examined, and 10% of the males. They were also more common in the forewings than the hindwings, in both sexes.

Delage-Darchen (1973) found that different species of *Crematogaster* showed vein instability in different regions of the wing. The variation I have found is all concentrated in a small area, except for the occasional appearance of an extra cu-a vein (see Figure 12.6). It may therefore be possible to identify other parts of the wing which are unstable in other colour forms of meat ants. A small collection of the yellow form I have examined indicates that at least some of the variation observed occurs in this form as well. Distinguishing between forms on this basis will therefore require the careful examination of large numbers of alates, which in most forms are not yet available. Wing vein variation therefore does not offer an immediate solution to the problem of distinguishing colour forms, although this conclusion may be modified when larger samples are collected.

Genitalia (usually of males) have also been a valuable source of diagnostic characters in many groups of insects. However, no description of the genitalia of meat ants has yet been published. Greenslade (unpublished) has found that there are small differences in the male genitalia of some combinations of colour forms, and at least one form (Dark Yellow) appears to be quite distinctive in this respect. These differences are consistent with the suggestion that the colour forms are different species, but once again, most of the differences are slight, and if considered in isolation, do not provide convincing taxonomic characters with which to separate every pair of forms.

CHAPTER 13

DISCUSSION AND CONCLUSIONS

It now seems clear that each of the eight currently recognized colour forms of meat ants constitutes a seaparate biological species. Strong evidence in favour of this conclusion is of three major kinds:-

a) Reproductive isolation between forms is indicated by the lack of gene flow for alleles at isozyme loci, particularly *Amylase* (see Chapter 7). Some combinations of forms cannot be distinguished on the basis of isozymes, and for others, the required data are not yet available. Nevertheless, the fact that some forms can be clearly shown to be reproductively isolated from each other, is *prima facie* evidence that the others are as well.

b) Some forms have sufficient differences in the male genitalia to justify their recognition as different species, and the Dark Yellow form appears to be particularly distinctive in this regard. The differences between some pairs of forms are slight, but will probably be found to be reliable.

c) The violent hostility which occurs between forms is in marked contrast to the ritualized aggression between colonies of the same form. Whatever the basis of this difference, it indicates that the ants themselves can recognize members of other colour forms as being somehow different from members of their own form.

Supporting evidence is also of several kinds, although less convincing than a-c above:-

d) With practise, the colour forms can be consistently and reliably distinguished on the basis of colour alone in almost all cases, and there is no evidence that they intergrade.

e) The colour differences between forms are often accompanied

by differences in nest type, and both small and large-scale examination of the geographic distributions of the various forms suggest consistent differences in habitat and climatic preference.

f) Finally, there may be small differences between some combinations of forms in morphology and karyotype.

Each of the points d-f if considered singly is not sufficient to clearly distinguish between forms on the basis of the data available at the moment. However when viewed in the light of the other evidence described above, they support the conclusion that the forms are indeed separate species. The con-commital term "forms" should be retained, however, until the species are formally named and described.

It is now possible to identify a series of characteristics which these forms possess, and to attempt to identify the ecological and evolutionary forces which may have contributed to their formation.

a) within each form, there is a very low level of genetic heterozygosity

b) the forms are very similar morphologically, genetically, and chromosomally

c) in areas where they are sympatric, their ecological requirements are so similar that they cannot peacefully co-exist

d) in various of the forms, there is geographic variation in allele frequencies, nest form, the complexity of colony structure, and perhaps in karyotype.

Very close similarity between the genomes of two species may indicate either that they have recently diverged from a common ancestor, or that they are subject to very similar selective regimes. Although these possibilities are not mutually exclusive, most of the evidence which is presently available argues in favour of the second alternative, and against the suggestion that meat ants are presently undergoing rapid

speciation.

The red and small purple forms have geographic distributions which could have arisen as a result of a recent speciation event (Figure 2.4). This may have involved a single very widespread ancestral species, whose range was reduced by a climatic shift to increasing aridity, resulting in the separation of eastern and western populations. These then evolved separately, forming the red and small purple forms respectively. Subsequent expansion by one or both forms may then have produced the area of overlap now found on Eyre Peninsula. The isolated colony of the red form at Alice Springs (Figure 2.4) is then seen as a relict of a once wider distribution, and populations of this form near inland watercourses may also have been produced in this way.

However, this situation is exceptional, and it is more common for forms to be extensively sympatric. The traditional model of allopatric speciation as employed above, involves the physical separation of two groups and their subsequent expansion so that contact is re-established. Reproductive isolation between them may evolve fortuitously, as a side effect of general changes in their genomes during the period of separation, or by selection against hybridization after they are re-joined. Whichever of these processes operate, a considerable time will probably be required for the two species to subsequently penetrate into each others' ranges. This may be especially true in meat ants, if each form saturates the available habitat, thereby reducing the opportunities for an invading form to become established.

The decorated multi-entrance mound nest built by some forms of meat ants, is not found in any other species of *Iridomyrmex*, nor indeed, in any other species of Australian ants. The complex behaviour patterns required for the building and occupation of such nests could probably be acquired only as the result of a prolonged period of evolution. Fur-

thermore, the distinction between the forms which live in these nests and those which usually do not, is accompanied by differences in size and genotype, so this distinction at least, seems to be of considerable age.

Further circumstantial evidence for the age of the colour forms comes from certain spiders (Zodariidae) which are both predators and mimics of meat ants. It is possible that these spiders gain an advantage in predation by mimicking their prey - that is, they are "aggressive mimics" (Wickler, 1968; Sazima, 1977). Several different species of spiders are involved, and different species are quite convincing colour mimics of the red, blue and black forms of meat ants. They are almost invariably found in association with ants of the appropriate colour (Greenslade, unpublished). The colour forms of meat ants have therefore been in existence for a sufficient time to allow specialist predators to develop, and for them to evolve the morphological adaptations appropriate to exploiting the variation in their prey.

The question arises then, if these forms are separate species, and if they have been separate for a long period of time, why have they not diverged from each other further than they have, either genetically or morphologically? To approach an answer to this question, it is necessary to consider what is known of their genetic structure, as well as the descriptions of their ecological background provided by Greenslade (1976a, and unpublished).

Hymenoptera species seem to be characterized by low levels of genetic heterozygosity (Chapter 9), and this was presumably the case for the single ancestral species from which the present meat ants evolved. This species may therefore have lacked the genetic variation required as the raw material for subsequent genetic divergence during speciation, and the present meat ants may have retained the genome of this progenitor

species in a fairly intact form. The low genetic distances between forms may also be caused in part by the overall low level of heterozygosity found. However, these genetic distances are only found to be abnormally low when populations of different colour forms are compared. The genetic distances between populations within a colour form are comparable to those found between geographic populations in other species of animals, despite the low level of heterozygosity within each population (Chapter 10). Therefore it seems plausible that the colour forms of meat ants have been prevented from diverging from each other any further than they have by some ecological factor or factors, in addition to whatever restriction is imposed by the overall lack of genetic variation.

In particular, meat ants are unusual among Australian ants in several ways. They are large, extremely numerous, and to some extent ecologically generalized, features which contribute to making them the dominant members of ant communities (Greenslade, 1976a). To maintain this position, they must be successful competitors, in an ant fauna which consists of at least 1500 species (Brown and Taylor, 1970), and probably as many as 3000. Many of these species are more specialized than meat ants, and have narrow niches which they exploit efficiently. One may speculate, then, that meat ants are subject to strong stabilizing selection for a uniform, well-integrated, successful genome. This genome provides the adaptations for broad ecological tolerance, high competitive ability, and dominance in communities, factors which differ only slightly between forms. Reinforcing the development of a uniform genome, may be other factors, such as the longevity of queens, and the avoidance of environmental extremes in the shelter of the nest, as discussed in Chapter 9, although these are features which are presumably common to many species of ants.

Superimposed on the uniform background genome are small differences between forms, in both genotype, and minor aspects of ecology. However, any large departure from the basic genotype, and the associated changes in ecology and behaviour, are almost certain to be in the direction of further specialization. Any such move towards specialization is unlikely to be successful, since it will bring meat ants into closer competition with one or more other species. These established species are capable of competing successfully with meat ants within their particular specialized niche. Furthermore, this specialization would narrow the range of resources available to meat ants, and further reduce their chances of success. The existing forms of meat ants may therefore be prevented from diverging from each other any more than they have, by pressure exerted on them from all sides by the large number of other species with which they compete - that is, they are subject to "diffuse competition" (MacArthur, 1972; Hebert *et al.*, 1974).

It would be of particular interest in this context to examine ecological and genetic data from other species which are closely related to meat ants. One such species is a rare, un-named ant presently known by the code name *Iridomyrmex sp. 24* (ANIC) (R.W. Taylor, personal communication; see Imai *et al.*, 1977 for an explanation of the nomenclature). This species, found in north-central Queensland, is morphologically very similar to the meat ants dealt with here, except for its possession of very large eyes. It is possible that that this species represents the result of a speciation event in which morphological divergence has progressed somewhat further than it has between the colour forms of *I. purpureus*.

Also, there are several species of *Iridomyrmex* which are smaller than meat ants, but otherwise very similar, and which often replace meat ants on sandy soils. Detailed examination of these species, as well

as *I.sp.24* (ANIC), could provide valuable insights into evolutionary trends in meat ants.

The fact that the colour forms of meat ants have not diverged very far from each other, makes it difficult to assess their historical relationships, and to formulate a simple series of speciation events consistent with their present distributions. However, there are several lines of research which could provide further relevant information if pursued in the future.

a) More extensive isozyme surveys. All the conclusions I have drawn from isozyme data could be strengthened by the incorporation of results from more loci. This is particularly true in the demonstration of reproductive isolation between more combinations of colour forms, and in the calculation of more reliable genetic distance estimates.

b) Large scale chromosome surveys. The existence of karyotype variation within forms means that a large body of evidence will be needed to convincingly demonstrate consistent differences between forms. In particular, geographically widespread populations within each form should be examined.

c) Multivariate analysis of morphological variation. It should be possible to measure a large number of variables in several populations of each colour form, to more accurately assess the extent of morphological variation. Also, the search should continue for distinct morphological differences between forms. For example, in meat ant workers, the proventriculus (a regulatory valve in the digestive tract) is elaborate and complex (Emery, 1913; Eisner and Brown, 1958; Wilson, 1971), and may provide a means of distinguishing between colour forms.

d) Examination of males and females. Both genitalia and wing veins have the potential of providing valuable taxonomic information if larger collections are examined. The specimens required could be

efficiently collected simultaneously with the larvae required for karyotype analysis as in b) above.

Much of the work in points a-d above would be very laborious and time consuming. This would probably be most rewarding in new areas not covered in the present survey. For example, in north Queensland, the red, black, dark yellow, and *sanguineus* forms can all be found close together, and the same is true of small purple, blue, orange and black in southern Western Australia.

Two other approaches may offer important information, however, and require somewhat less labour:-

e) Behavioural differences between colour forms. It should be possible to locate areas of close sympatry between further pairs of colour forms, to look for hostile interactions between them. For example, we can predict that violent hostility should occur between adjacent colonies of blue and black, yellow and red, small purple and red, and small purple and yellow. These pairs of forms can all be found close together on Eyre Peninsula.

f) Identification of pheromones. Many aspects of ant behaviour are influenced by chemical communication (*e.g.*, Wilson, 1971). The identification of the substances involved (perhaps by gas chromatography) could provide valuable information about the differences between colonies of the same colour form, as well as identifying possible differences between forms. Also, it is likely that mating is stimulated by sex pheromones released by males or females, or both. Analysis of these substances could provide information different in kind from anything which has been considered to date, and may offer vital clues as to how the colour forms of meat ants can be reproductively isolated, while remaining very similar in almost all aspects of their biology.

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APPENDIX 4

Esterase variation at three loci in meat ants, Iridomyrmex purpureus.

R. B. Halliday.

SUMMARY

The meat ant (*Iridomyrmex purpureus*) occurs in a number of colour forms, of uncertain taxonomic status. Gel electrophoresis of meat ant extracts, followed by non-specific esterase staining, reveals several zones of activity. Allelic variation at three loci is proposed to account for variation in some of these zones. Two of the loci (*Es-1*, *Es-2*) appear to have recessive null alleles, whose frequencies have been estimated by the method of maximum likelihood. Geographic variation in allele frequency is attributed to behavioural and geographic subdivision of the population. Apparent disturbances in segregation ratios and deviations from Hardy-Weinberg equilibrium seem to be caused by the presence of more than one queen in some nests. Differences in gene frequency between sympatric populations of the red and blue forms of *I. purpureus* are observed, confirming their reproductive isolation and sibling species status.

INTRODUCTION

Meat ants (Iridomyrmex purpureus Smith) are a conspicuous element of the Australian ant fauna, and have long been known to consist of a number of forms, which differ in colour, geographic distribution and nest structure. [4,6,7,8]. Five of these colour forms are included in this study. They are "red" (purpureus sens. strict.), "black" (viridiaeneus Viehmeyer), "blue"[6], and the previously unreported "yellow" and "small purple" forms. The distribution of the red and blue forms in South Australia is described by Greenslade[7,8]; details of the appearance and distribution of the other colour forms will be described in forthcoming publications. Colonies of the red, black and yellow forms consist of one or more low, gravel covered mounds with numerous entrance holes. In the blue form and at least in South Australia, in the small purple form, colonies are composed of scattered single-entrance nests, sometimes with turrets, but without gravel decoration.

A study of electrophoretic variation at an amylase locus (Amy) indicated that some of these colour forms may actually be sibling species[10]. The gene frequencies of the co-dominant amylase alleles were estimated by typing a number of workers from each nest, and from their genotypes, inferring the genotypes of the queen and male which founded the nest.

The esterase variation described in this paper seems to include the segregation of recessive "null"alleles. These are well known for esterase loci in insects e.g., Lepidoptera[1], Diptera[14], and Hymenoptera[2], although it is not known to what extent lack of activity on an artificial substrate in vitro reflects a real lack of in vivo activity. The estimation of the frequencies of these alleles is more difficult than for co-dominant alleles, since they cannot be obtained by direct gene counting. The

social behaviour of ants adds further complications to this process, and the frequencies of the null alleles examined here have been estimated using the method of maximum likelihood.

MATERIALS AND METHODS

Figure 1 shows the areas in South Australia where ants were collected, and indicates place names referred to later. The climate in this area ranges from subhumid in the south to arid in the north. Worker ants were taken from the surface of the nest or its immediate vicinity, and males were collected from some nests by excavation. Representative voucher specimens will be lodged with the Australian National Insect Collection, CSIRO Division of Entomology, Canberra. Ants were killed by freezing and stored at -30°C until used. The abdomen was discarded, and the head and thorax homogenized in 3 drops of distilled water. Samples were then centrifuged for 1 minute in a Beckman 152 Microfuge and used immediately.

Horizontal starch gel electrophoresis was carried out at 3°C in a Tris citrate/Lithium borate buffer system at pH 8.5[3]. The staining mixture for 32 samples contained 50 mg. of α- and 50 mg. of β- naphthyl acetate (pre-dissolved in a few ml. of acetone), 50 mg. of Fast Garnet GBC salt, 50 ml. of methanol, and 50 ml. of Tris/maleic acid buffer pH 6.0. At room temperature bands of esterase activity became visible in a few minutes and could be scored within an hour.

RESULTS

Gels stained in this way reveal a complex array of bands similar to that found in the harvester ant Pogonomyrmex barbatus[11]. Up to 6 zones of activity can be seen in some specimens, and several of them show variation (Fig. 2). Three gene loci are proposed to account for the

variation in some of the most distinct bands.

Esterase-1

The slowest anodal band of activity is always either clearly present or clearly absent. This variation is attributed to the locus Esterase-1 (Es-1). Worker ants can be classified as either "+" or "null" with respect to their Es-1 phenotype, and nests contain workers which are either "all +", "all null", or of both types. In practice, a minimum of six (usually more) workers of the same phenotype have been scored from a nest, before it is calssified as either "all+" or "all null". Table I shows results from all nests in which both workers (diploid) and males (haploid) have been scored, and the mating required to produce the results in each case. These data are consistent with the suggestion that the phenotypes are controlled by a recessive null allele Es-1^{o} and an active allele Es-1^{+}.

If this genetic model is correct, and if there is one singly-mated queen per nest, any nests which segregate for Es-1 are expected to show a 1:1 ratio of +:null workers. This would be produced by the mating Es-1^{+}Es-1^{o} x Es-1^{o}. Also, the distribution of segregation ratios ought to be binomial, with equal numbers of ants of the two phenotypes. This has been tested in the 40 known segregating nests of the red form, using 10 randomly selected workers from each. This sample contains 195+:205 null workers. However, Table II shows that the observed and expected distributions of segregation ratio differ significantly ($\chi^2_6 = 23.73$, $P<0.001$), and that the observed distribution is rather irregular and over-dispersed. This result is most easily interpreted as a partial failure of the assumption of one singly mated queen per nest. Both the results obtained

from the Amylase locus,[10] and excavation of nests[5], also suggest that some nests contain more than one queen, which complicates the problem of gene frequency estimation. However, if it is assumed that there is a single queen per nest, and that the population is in Hardy-Weinberg equilibrium, it is possible to derive the expected frequencies of the three types of nest (Table III). Approximate gene frequencies can then be obtained by the method of maximum likelihood. If the observations in a population are J nests with workers "all null", K with segregating workers, and M with workers "all +", then the logarithm of the likelihood of a particular value of q is given by

$$L = \text{Constant} + J\log q^3 + K\log(2q^2 - 2q^3) + M\log(1 - 2q^2 + q^3) \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where q is the frequency of the null allele Es-1^o.

Differentiating this, and simplifying, gives

$$\frac{dL}{dq} = \frac{3J}{q} + \frac{2K - 3Kq}{q - q^2} + \frac{3Mq^2 - 4Mq}{1 - 2q^2 + q^3} \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

The estimated value of q for a population is then obtained by inserting the observed values of J,K, and M, and solving equation (2) when dL/dq = 0. This process reveals one and only one value of q for each population, as shown in Table IV. These results include only the red form of mediants, since Es-1+ occurs only rarely or not at all in the other forms studied. Standard errors of q have been derived from the second differential of the likelihood expression, using the relationship[12]

$$\frac{d^2L}{dq^2} = \frac{-1}{\text{Variance of } q} \quad \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

When a value of q is obtained, it can be substituted into the expected frequencies of the three nest types, to test agreement with the observed data. The expected figures given in Table IV show that this agreement is usually close.

The gene frequencies of Es-1^{o} shown in Table IV reveal highly significant heterogeneity among populations of the red form (G = 50.46, P<0.001). Also, many of the possible pairwise comparisons of populations show significant differences in gene frequency. The geographic variation observed for the frequencies of esterase alleles in the harvester ant had a principal component associated with "weather"[11]. However, there is no evidence of a simple association between Es-1^{o} frequency and climate in the meat ant populations studied here. The "Adelaide" and "Eyre Peninsula" populations are among the most similar with respect to gene frequency, but among the most divergent climatically (i.e., subhumid vs. semi-arid.

Table V shows observed numbers of the three nest types for Es-1 in forms of meat ants other than red. With three exceptions, the populations appear to be fixed for the null allele Es-1^{o}. In two cases, nests of the blue form contain workers of the + phenotype. These workers are in a minority in both cases - 2 out of 24, and 2 out of 19. Colonies of the blue form often consist of a number of scattered entrance holes, and it is quite possible that such a colony could contain more than one queen. The existence of rare Es-1^{+} alleles in this form would then produce segregation ratios similar to those observed. The figures shown in Table V for the Morgan population of viridiaeneus (V) give a frequency for Es-1^{o} of 0.924± 0.042, which is not significantly different from that for the sympatric population of the red form (P≈0.2). The difference in Es-1^{o} frequency between sympatric red and blue form populations is significant at Dublin (P<0.001), Eudunda (P<0.001), and Morgan (P<0.01).

Esterase-2

The second slowest zone of esterase activity also shows presence/absence variation (Fig. 2). This is attributed to two alleles at the

Esterase-2 locus, Es-2^{o}, and Es-2^{+}. While the mode of inheritance of Es-1 phenotypes was confirmed by the finding of Es-1 null males in some nests (Table I), males with the null phenotype for Es-2 have not been observed. Consequently the existence of the recessive null allele Es-2 remains unconfirmed.

Specimens with the null phenotype at this locus have only been observed in the blue form of meat ants, and only in a total of 8 nests. At Dublin, 3 nests segregate for Es-2 out of a total of 17, which gives a gene frequency for Es-2^{o} of 0.303±0.089, derived in the same way as Es-1^{o} frequencies. At Morgan, one nest out of 18 contains workers all with the null phenotype for Es-2, and two others segregate, giving q = 0.330± 0.088. In both these populations the Es-2 null workers are in a minority in the nests which segregate, (total 86+:21 null), again suggesting polygyny This means that the gene frequencies for Es-2^{o} given are overestimates of its true frequency. The Es-2^{o} allele also occurs in a small sample of the blue form from Hallet Cove, so it appears to be quite widespread.

Data from colour forms other than blue are not sufficient to rule out the presence of Es-2^{o}. The Dublin red form population contains 17 nests, none of which have any Es-2 null workers. This gives an upper limit of 0.198 to the true frequency of such nests, corresponding to a value of 0.346 for the upper limit of the frequency of Es-2^{o} (at the 95% level[13]). Thus, gene frequencies at this locus cannot be used as evidence of reproductive isolation between colour forms.

Esterase-3

The specimens shown in Fig 2 all have a single band of Esterase-3 activity (Es-3^{a}Es-3^{a}). Others have a single slower band or both bands, in the pattern characteristic of a pair of co-dominant alleles. Neither of the bands overlaps with the products of genes at other loci, so all three

genotypes can be recognized. The slow allele Es-3^b has only been observed in two nests of the red form, close together at Eudunda. One of these shows the expected 1:1 segregation of Es-3^{a}Es-3^a and Es-3^{a}Es-3^b workers produced by the mating Es-3^{a}Es-3^b x Es-3^a (observed numbers 9:11). The other nest contains 22 Es-3^{a}Es-3^a : 12 Es-3^{a}Es-3^b : 5 Es-3^{b}Es-3^b which cannot be produced by one singly-mated queen. This nest also shows aberrant segregation ratios for the Es-1 and Amy loci, so polygyny is again strongly suggested.

Other Loci

Most specimens show some extra zones of esterase activity in addition to those which have been attributed to Es-1, Es-2 and Es-3. These additiona zones appear both between the Es-2 and Es-3 isozymes, and anodal from Es-3. However, they do not stain consistently with present techniques, so they are not considered further. Also, there is some quantitative variation in the relative strengths of some zones, but this does not interfere significantly with the scoring of phenotypes, and is probably non-genetic in origin.

DISCUSSION

The gene frequency estimation procedure used here has involved a number of assumptions. Firstly, it is assumed that the gene frequencies in males and females are the same. This is difficult to test, but a persistent difference in gene frequencies could only be produced by differential selection. Although male and female ants differ in many ways, it is difficult to see how differential selection at the loci described here could be strong enough to produce detectable changes in the frequencies of nest types.

Secondly, it is assumed that the population is in Hardy-Weinberg

equilibrium. Zouros and Krimbas[14] also found it necessary to make this assumption when using maximum likelihood to estimate null allele frequencies in a fruit fly Dacus oleae. Although the genotype frequencies among reproductives in meat ants cannot be examined directly, it is possible to compare the observed and expected frequencies of the three nest types. Table IV shows that the agreement between these is usually close, except for apparent excesses of segregating nests at Dublin and Morgan. However, rather than indicating a deviation from Hardy-Weinberg frequencies, this is just what would be expected if some nests contained more than one queen.

The third assumption of the method, then, is that each nest contains one singly-mated queen, and there is some evidence which suggests that this is not always the case. Also, some meat ant colonies consist of a number of separate nests which exchange workers freely. The presence of several queens of differing genotype in such a colony, would give the appearance of polygyny in a sample of workers collected from a single nest. Polygyny or multiple insemination would cause the distribution of segregation ratios to be skewed, with the extent and direction of the skewness depending on the gene frequencies in the particular population studied. The unusual distribution shown in Table II has probably been produced by pooling results from populations which vary in gene frequency, and by the presence of some polygynous nests and multi-nest colonies. An unknown level of polygyny introduces some uncertainty into the gene frequencies obtained by this method. Some of this could be resolved by extensive excavation of nests, to allow measurement of the level of polygyny and direct measurement of the gene frequencies in reproductives. However, the labour and population destruction required to not seem justified at present.

Despite these uncertainties, the results obtained here seem to be the best estimates of gene frequency available at the moment for loci

with dominance in social Hymenoptera.

The method applies specifically to a locus with a null allele and a single active allele. Where the null allele is accompanied by a series of active mobility variants, a two-stage procedure is used, with the frequency of the null allele estimated first[2]. These two procedures yield the same result for the frequency of the null allele, but the maximum likelihood method has the advantage of providing a variance estimate as well as the gene frequency.

Evidence supporting the model proposed for the genetic control of Es-1 phenotypes was obtained by typing males (Table I). However, expectations analogous to those shown in Table III can be derived, and allele frequencies estimated, for a dominant Es-1^{o} in the same way as for the recessive. The expected numbers of nest types produced by the two models agree with the observations equally well. Thus, the procedure described here for gene frequency estimation must be based on prior knowledge of the mode of inheritance of the phenotypes, which cannot be obtained purely from population data on workers.

Another possibility is that the enzymes which I have attributed to the loci Es-1 and Es-2 are actually produced by mobility alleles at a single locus. The slow homozygote at such a locus would have the phenotype Es-1 +, and Es-2 null. The expected frequency of nests containing workers of this phenotype can be calculated for each population, since the fast allele at this locus would have the same gene frequency as that calculated for Es-1^{o}. The 6 populations in Table IV give a total expectation of 15.7 such nests. Adding a null allele to this hypothetical locus also produces workers with this phenotype, but in this case their frequency cannot be simply derived. The fact that no nests containing workers of this type were found, argues against the validity of this model.

The observed geographic variation of Es-1 allele frequencies in the red form of meat ants has probably been produced by a combination of geographic and behavioural subdivision of its range. Fig. 1 shows that the Eyre Peninsula and Morgan populations are somewhat isolated from the main body of its distribution in the Mount Lofty Ranges. This tendency towards fragmentation of range is likely to be reinforced by the species' reproductive behaviour. The mating flights of this form only occur on clear, still afternoons, with the temperature close to 21°C, and the ground wet from recent rain[5]. These conditions may be limited in extent so further subdivision of the population and a degree of local inbreeding are likely to result. Spatial variation in gene frequency, then, could be produced by a combination of founder effects and drift in relatively small local populations.

Comparisons of Es-1 allele frequencies between some combinations of sympatric colour forms reveal significant differences. This is consistent with the earlier finding that some combinations of forms are reproductively isolated, and should be regarded as sibling species[10]. This is especially true of the red and blue forms, which differ significantly in Es-1 frequencies in almost every area where they occur together. As with Amylase, however, the other colour forms cannot be clearly distinguished, and other types of evidence are being examined to determine their relationships.

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Table I. Meat ant nests in which both workers and males have been typed for Esterase-1. Allele notation abbreviated; o = Es-1^{o}, + = Es-1^{+}.

Nest number	Workers		Males		Inferred mating
	+	Null	+	Null	
98	6	0	0	6	oo x +
131	3	8	3	5	+o x o
134	0	8	0	8	oo x o
201	7	13	1	1	+o x o
212	0	10	0	8	oo x o
228	0	8	0	8	oo x o
234	17	13	19	8	+o x o
235	11	0	5	6	+o x +
236	0	8	0	8	oo x o
237	0	8	0	5	oo x o
408	8	0	5	3	+o x +
418	17	0	2	6	+o x +
425	6	5	1	2	+o x o
455	10	0	8	0	+? x +
456	10	0	8	0	+? x +

Table II. Observed and expected distribution of segregation ratios for Es-1 in 40 families of size 10.

Number of + workers	Number of nests: Expected		Number of nests: Observed		Direction of deviation
10	0.039		0		
9	0.391	2.188	1	4	+
8	1.758		3		
7	4.688		5		+
6	8.203		10		+
5	9.844		4		-
4	8.203		8		-
3	4.688		1		-
2	1.758		5		
1	0.391	2.188	2	8	+
0	0.039		1		

Table III. Observed and expected frequencies of the three nest types produced by all possible matings at the Esterase-1 locus, in terms of q, the allele frequency of Es-1^{o}. Allele notation abbreviated as for Table I.

Mating	Worker genotypes	Worker phenotypes ("nest type")	Expected frequency	Expected frequency of nest type
oo x o	oo	All null	q^3	q^3
+o x o	½+o:½oo	Segregating	$2q^2 - 2q^3$	$2q^2 - 2q^3$
++ x +	++	All +	$(1 - q)^3$	$1 - 2q^2 + q^3$
++ x o	+o	All +	$q(1 - q)^2$	
+o x +	½++:½+o	All +	$2q(1 - q)^2$	
oo x +	+o	All +	$q^2(1 - q)$	

Table IV. Gene frequency (q) of Es-1^{o} in populations of the red form of meat ants. Expected number of nests in brackets below observed number.

Population	Number of Nests			q±SE
	Workers all+	Workers segregating	Workers all null	
Dublin	7 (9.26)	9 (4.71)	1 (3.03)	0.56±0.08
Eudunda	10 (10.15)	6 (5.72)	4 (4.13)	0.59±0.07
Arthurton	8 (8.79)	7 (5.57)	4 (4.64)	0.63±0.07
Morgan	0 (3.04)	8 (3.70)	8 (9.26)	0.83±0.05
Adelaide	1 (1.11)	2 (1.87)	15 (15.02)	0.94±0.03
Eyre Peninsula	0 (0.34)	1 (0.63)	12 (12.03)	0.97±0.03

Table V. Esterase-1 nest types in non-red colour forms.

Place names and abbreviations as in Fig. 1.

* see text

Population and colour form	Number of nests: Workers all+	Workers segregating	Workers all null
Hallett Cove (B)	0	0	4
Eyre Peninsula(B)	0	0	10
Morgan (B)	0	1*	17
Flinders Ranges (B)	0	0	7
Snowtown (B)	0	0	4
Eudunda (B)	0	0	17
Dublin (B)	0	1*	16
Eyre Peninsula (V)	0	0	5
Morgan (V)	2	1	12
Flinders Ranges (V)	0	0	8
Eyre Peninsula (SP)	0	0	5
Mundoora (SP)	0	0	4
Eyre Peninsula (Y)	0	0	6

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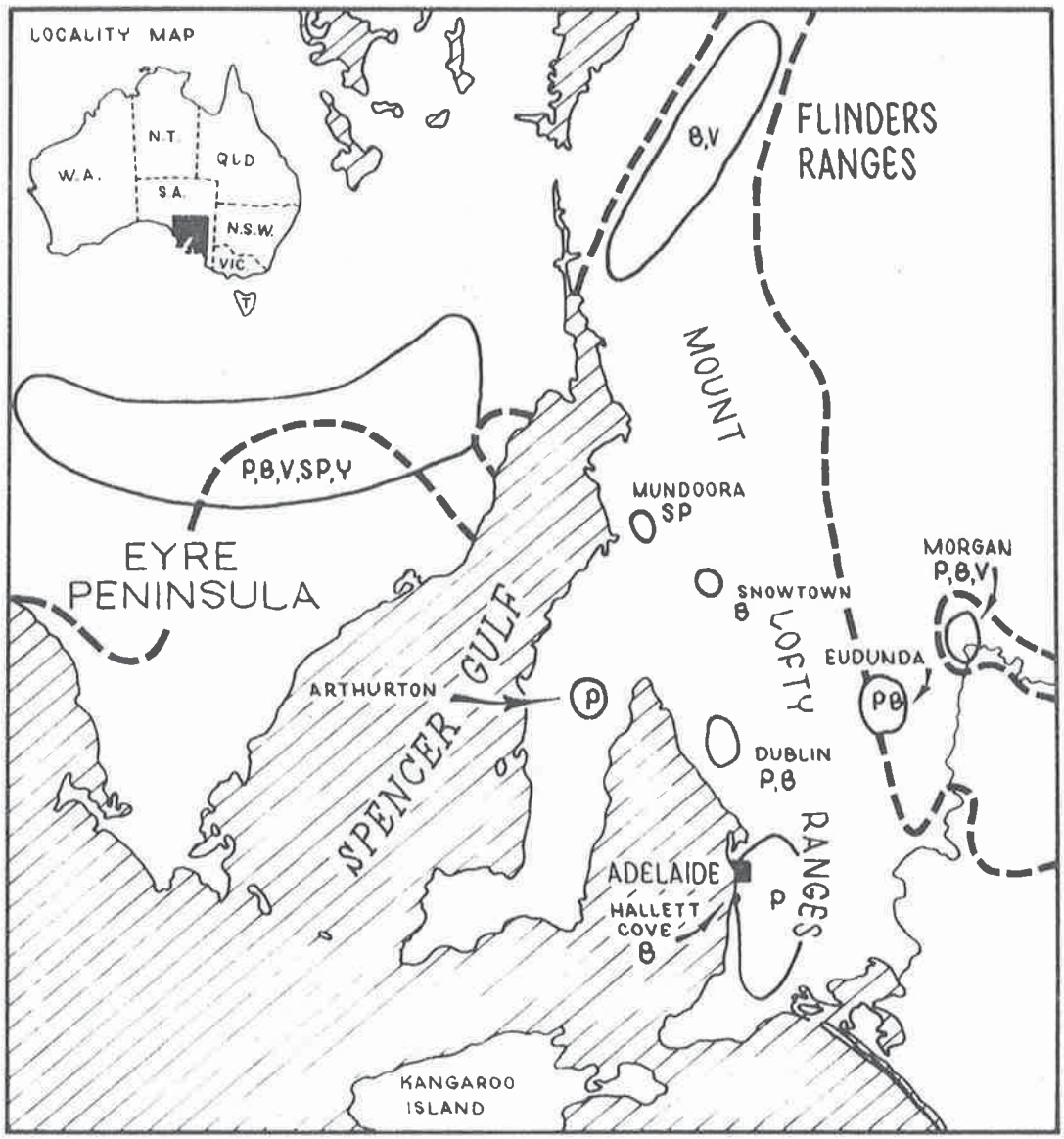

Fig. 1. Place names referred to in text and areas sampled. Colour forms collected from each area indicated. P = red form (*purpureus* sens.strict.), B = blue form, V = black form (*viridiaeneus*), SP = small purple form, Y = yellow form. Solid lines enclose areas sampled. Broken line shows distribution limits of P.

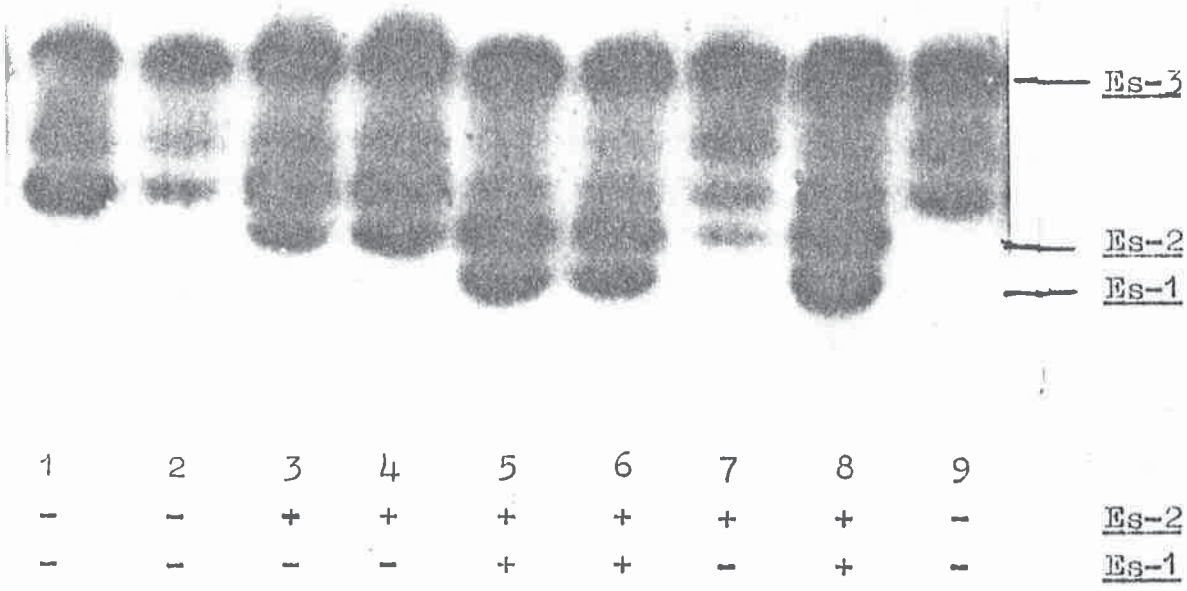

Fig. 2. Photograph of a gel stained for esterase activity, with Es-1 and Es-2 phenotypes indicated. Direction of migration is upward.

APPENDIX 5

Abbreviations.

a) Colour forms of meat ants

P = Red form (*purpureus sens. strict.*)

B = Blue form

V = Black form (*viridiaeneus*)

Y = Yellow form

SP = Small Purple form

DY = Dark Yellow form

S = *sanguineus*

O = Orange form

b) Chemicals and reagents

TEMED	= N,N,N',N'-Tetramethyl -ethylenediamine
Tris	= Tris(hydroxymethyl)aminomethane
HCl	= Hydrochloric acid
MTT	= 3-(4,5-Dimethyl Thiazolyl-2)-2,5-diphenyl Tetrazolium Bromide
PMS	= Phenazine methosulphate
GBC	= O-Amino Azotoluene, Diazonium salt
$CaCl_2$	= Calcium Chloride
NADP	= β-Nicotinamide adenine dinucleotide phosphate
Na_2 EDTA	= Ethylenediaminetetraacetic acid, disodium salt
NAD	= β-Nicotinamide adenine dinucleotide.

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