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**DIFFERENTIATION OF SIBLING SPECIES IN THE ANT GENUS APHAENOGASTER:
KARYOTYPIC, ELECTROPHORETIC, AND MORPHOMETRIC INVESTIGATIONS
OF THE FULVA-RUDIS-TEXANA COMPLEX**

by

GARY JOHN UMPHREY, B.Sc., M.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of

Doctor of Philosophy

Department of Biology

Carleton University

Ottawa, Ontario

1 May, 1992

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for the degree of Doctor of Philosophy

Chair, Department of Biology

Thesis Supervisor

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1 June, 1992

ABSTRACT

The fulva-rudis-texana complex of the ant genus Aphaenogaster Mayr includes A. fulva Roger, A. rudis (Emery), and A. texana (Emery), and morphologically similar species. Karyotypic, electrophoretic, and morphometric investigations were conducted into the systematics of this complex, with the primary goal of delineating the component species in eastern North America. Ten sibling species are recognized, some provisionally, on the basis of karyotypic and electrophoretic markers. Nine distinctive basic karyotypes can be recognized. Two species that have similar $n = 22$ karyotypes can be separated electrophoretically. Provisionally, the following are valid names for six genetically delineated species: A. fulva, A. rudis, A. texana, A. carolinensis Wheeler, A. miamiana Wheeler, and A. picea (Emery); the other four species are undescribed. In addition, morphological evidence suggests that A. punctaticeps MacKay is a valid species, but A. huachucana crinimera Cole is a synonym of A. texana.

Basic karyotypes show considerable variation among species in chromosome number ($n = 16 - 22$) and chromosome morphology. Karyotype evolution within the complex has proceeded primarily via Robertsonian changes. Karyotype variation within species was mainly due to the occasional presence of supernumerary chromosomes. Some supernumeraries were found in somatic (cerebral) tissue, whereas others were restricted to the male germ line.

A model is presented suggesting that, under certain conditions, interspecific mating by queens of the eusocial Hymenoptera with the subsequent production of infertile daughter offspring can be selected for as part of a mixed evolutionarily stable strategy. Electrophoretic and chromosomal evidence confirms the hybrid nature of one colony found in this study.

Morphometric methods, including discriminant analysis and canonical variate analysis, are used to examine the degree of morphological differentiation among species. Karyotyping is

the most reliable method for identification of species in the complex, but it is not sufficient in itself. This study provides a sound basis for further systematic research in Aphaenogaster.

To family and friends,
but especially to my wife,
Jennifer.

Three down,
none to go.

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I would like to thank Henry F. Howden, for the opportunity to complete my doctorate programme under his supervision. Many years ago, while working as an undergraduate in the laboratory of D. H. Pengelly at the University of Guelph, I became fixated on ants as magnificent organisms worthy of lifelong study. The goal of obtaining a Ph.D. via myrmecological research has been an important intermediate objective in my career ambitions. Henry's advice, financial support, encouragement, and unfailing patience have all been important factors in helping me to achieve my objectives. The dedication of Henry and his wife Anne to the study of the Coleoptera has been a source of inspiration; it is rather a shame that they didn't work on ants! Jennifer and I greatly appreciate the many kindnesses we have received from the Howdens over the years, and wish them many more years of great beetle hunting.

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1. INTRODUCTION AND REVIEW

1.1. Introduction

By virtue of their numbers, biomass, impact in terrestrial ecosystems, wide geographical distribution, and complexity and diversity of social organization, ants rank as one of the most important groups of animals. However, they are only modestly diverse on a taxonomic basis relative to their abundance. Constituting the single family Formicidae in the order Hymenoptera, only about 8800 valid species have been described in the 297 extant genera recognized by Hölldobler and Wilson (1990); they estimate that if the taxonomy of the world ant fauna is fully investigated 20,000 or more species may exist in as many as 350 genera.

The best known ant fauna on a broad regional basis is that of North America north of Mexico, largely due to W. S. Creighton's monumental work, "The Ants of North America", published in 1950. Since then, most large (and many smaller) Nearctic ant genera have received further revisionary treatment (see Hölldobler and Wilson, 1990, for a list of such revisions). One genus that has not been revised, despite the acknowledged need (Carroll, 1975; Smith, 1979) is Aphaenogaster (first described by Mayr, 1853). The key to revising the North American Aphaenogaster lies in the resolution of what I call the "fulva-rudis-texana complex". This is a set of morphologically similar species that meet at least one of the following criteria: (i) fulva Roger, rudis (Emery), and texana (Emery) are included; (ii) species described as intraspecific forms of fulva, rudis, or texana are included; (iii) species that have been considered closely related to at least one of fulva, rudis, or texana are included. By calling this a complex I do not wish to imply that it represents a monophyletic group (although it probably is) but rather that it represents a group of species for which the species have proven difficult to define and delimit using morphological criteria. Section 1.2 discusses the composition of this complex and reviews its systematic history.

Members of the fulva-rudis-texana complex are some of the most abundant ants in the forests of eastern North America, where they appear to reach their maximum diversity. Their combined range is from the Florida Keys north to southern Ontario and Quebec, west to Colorado and the mountains of Arizona, and south into Mexico. They have a monomorphic worker caste (like ?! Aphaenogaster) and are general predator-scavengers feeding primarily on other insects. They also feed on the eliasomes of myrmecochorous seeds and are one of the most important dispersal agents for many woodland herb species (Beattie, 1985). Mature colonies usually have a single queen (but not invariably; see Headley, 1949; Talbot, 1951) and up to several hundred workers (Carroll, 1975; Headley, 1949; Talbot, 1951). Nests are usually found in rotten wood, in soil with or without a covering object, or in leaf litter.

At the most basic level, the purpose of my thesis research has been to make systematic sense out of the fulva-rudis-texana complex. Some preliminary work of my own, the pioneering studies of Crozier (1975, 1977), and the analysis of the Florida forms by Carroll (1975) convinced me that I was unlikely to achieve a satisfactory degree of resolution using standard morphological procedures as my only methodology. Furthermore, Crozier's work (discussed in detail in section 1.3) showed that the genetic techniques of karyology and electrophoresis would be highly informative for this complex. Since these procedures were considered efficient in terms of cost and time, and methodological details were reasonably well established for ants (see sections 1.4 and 1.5), both procedures were used in this study. Emphasis has been placed on identifying genetic markers which would delineate the component species of this complex. By building a collection of specimens from genetically identified colonies, morphological differentiation could be studied with greater confidence in the initial delimitation of the species. Hopefully, simple external characters would be found that would allow routine morphological identification of the ants in this complex. Failing this, various morphometric approaches

(particularly discriminant analysis) could be used to try to optimize classification from measurements of external characters. These analyses would also indicate why the species in the complex were so difficult to separate morphologically. It has, in fact, proved necessary to follow this line of investigation. A brief rationale for the morphometric approach is presented in section 1.6.

A single ant colony can supply sufficient material for karyotypes, electrophoresis, and morphological study (and other procedures as well, if so desired). Hence the concordance of the different methodologies can be compared with greater confidence than when the different methods are performed on different individuals of uncertain relationship. Concordance of results and the relative effectiveness and efficiency of the different procedures for solving problems in ant systematics are addressed in section 3.16.

Three questions are of obvious interest in a systematic study using genetic methodologies, and all are addressed in this thesis. First, is there any evidence of gene flow or at least the breakdown of reproductive isolating mechanisms between putative species? Secondly, if genetic markers are going to be used to delineate species, then what is the nature of complicating factors, such as supernumerary chromosome variation? Thirdly, are any of the genetic characters useful indicators of phylogenetic affinity?

Species concepts and definitions have been discussed at considerable length in the literature (see, for example, reviews by Templeton, Cracraft, and Nelson in Otte and Endler, 1989). Barton and Hewitt (1985) have discussed a fundamental dichotomy in the interpretations of the biological species concept of Mayr (1942). A strict interpretation (such as that of Key, 1981, discussed further in section 3.10) allows no gene exchange whatsoever between species (although infertile F_1 hybrids can be formed). Less strict interpretations allow some degree of introgression and/or further hybridization between species (e.g., Mayr, 1982; see also the

cohesion species concept in Templeton, 1989). Barton and Hewitt (1985) favoured a strict species definition that requires complete reproductive isolation. Under such a definition, the phrase "introgression between species" and the title "Drosophila hybrids in nature: proof of gene exchange between sympatric species" (Carson *et al.*, 1975) are oxymorons. However, Templeton (1989) has pointed out difficulties with the application of a strict species concept. Among examples he cited, balsam poplars and cottonwoods (both in the genus Populus) have been distinct lineages for at least 12 million years yet still produce fertile hybrids, and wolves and coyotes occasionally produce fertile hybrids. In insects, numerous examples of introgression have been recorded between morphologically distinct "species" (e.g., in Drosophila by Carson *et al.*, 1975; in the gerrid genus Limnoperus by Spence, 1990). Undoubtedly many more examples will be demonstrated with the advent of modern molecular techniques. I believe a strict species definition, although objective, suffers from a problem of verifiability. Although the hypothesis of complete reproductive isolation can be falsified, it is experimentally unverifiable, as the failure to falsify the hypothesis does not verify it.

Mayr (1969) noted that the first step in a morphological revision of a taxonomic group is to sort the available specimens into "phenotypically reasonably uniform samples", for which he used the term "phena" (singular "phenon", not used in the same sense by Sneath and Sokal, 1973). A single species may contain more than one phenon, due to such phenomena as sexual differentiation, castes, and polymorphic races. A single phenon may also contain more than one sibling species. The fulva-rudis-texana complex is essentially such a sibling species complex. A primary objective in my research has been to separate the samples into "genetically reasonably uniform samples"; for such a sample one could coin the term "genon" (I have lumped males, queens, and workers with the same genetic identity into the same genon, although they could be placed into separate gena if one wanted to be consistent with Mayr). Such "gena" may represent

separate species, but they might also represent such phenomena as subspecies, semispecies, chromosomal races, or genetic polymorphisms. The status of each genon is evaluated by searching for evidence of reproductive isolation from the other gena.

In this thesis, I have used the informal term "form" to represent such gena. The use of the term "form" has a long tradition in ant systematics; it was used extensively by Creighton (1950), for example. This may be due to the quadrinomial system of nomenclature used by myrmecologists from about 1875 to 1950 (Creighton, 1950) which current ant taxonomists are still trying to unravel. Creighton (1950) showed that the application of the infraspecific categories of subspecies and variety were highly inconsistent, eliminated the variety as a category deserving a latinized name, and followed Mayr (1942) in treating subspecies as geographical races. The suggestion of Wilson and Brown (1953; see also Brown and Wilson, 1954) that the subspecies be eliminated as a formal taxonomic category has since been followed by most ant taxonomists. However, the use of the term "form" has continued to have utility in ant systematics. Searching for evidence of reproductive isolation (or lack thereof) between species is rather contradictory; the utilization of "form" eliminates such contradictions while allowing the evidence for separate species status under different species concepts to be judged on its own merits.

Ants, like all other Hymenoptera, are haplodiploid. Ants are ideal haplodiploid organisms for addressing basic questions in systematics and population genetics since they are abundant, generally are less obnoxious to work with than social wasps and bees, and have a diversity of mating systems and social systems. Hamilton (1964a,b) recognized the central role the eusocial Hymenoptera could take in testing his theories of kin selection, and hypothesized haplodiploidy itself as a primary factor in the evolution of eusociality in the Hymenoptera. Trivers and Hare (1976) combined Hamilton's kinship theory with Fisher's (1930) sex ratio

theory to advance a theory of sex ratio investment in social Hymenoptera. They used ants as their primary source of data for testing their theory, as have numerous subsequent workers (e.g., Boomsma, 1990; Boomsma and Grafen, 1990; Boomsma and Isaaks, 1985; Nonacs, 1986a,b; van der Have *et al.*, 1988).

In section 3.15 I advance a hypothesis that under certain conditions interspecific mating by queen ants with the subsequent production of infertile female offspring may constitute part of a mixed evolutionarily stable mating strategy, rather than representing a simple case of "breakdown of reproductive isolating mechanisms". Haplodiploidy and eusociality are key components to this hypothesis: the queen will produce pure males of her own species, and thus the question becomes whether the loss in fitness caused by producing infertile reproductive females can be compensated for by possible superiority of the hybrid workers under certain conditions, particularly in marginal habitats. The model for the evolution of "sperm parasitism" is, I believe, the first in biology which suggests that natural selection could favour interspecific hybridization under a strict species definition.

A key objective was to place the results of Crozier (1975, 1977) into a geographically broader context. Furthermore, it was unclear how Crozier's results fit into the existing taxonomic framework. Obviously it has been necessary to address the issue of how existing names apply to the genetically recognized forms in the fulva-rudis-texana complex. Due to time and cost considerations, it was decided initially to limit the thesis research geographically to eastern North America. However, thanks to certain people who sent me live Aphaenogaster colonies from more distant localities (see section 2.1), I have been able to expand the geographic and taxonomic scope of the research further than originally intended. Their efforts have made a significant improvement in the value of this work.

1.2. A Systematic Review

Members of the genus Aphaenogaster are moderate-sized to fairly large myrmicine ants. They have a broad geographical distribution but are apparently absent from South America and sub-Saharan Africa. Aphaenogaster is one of nineteen genera, along with Pheidole Westwood and Messor Forel, included in the tribe Pheidolini by Hölldobler and Wilson (1990). [Kartidris, recently described by Bolton (1991), represents a new addition to the tribe.] The workers of Aphaenogaster are monomorphic and have a characteristic "sway-backed" alitrunk, but they can be difficult to identify to genus. In most keys to myrmicine genera, Aphaenogaster appears at or near the end of the key (e.g., see keys in Hölldobler and Wilson, 1990). Indeed the monophyly of Aphaenogaster has not been established; all five characters that Bolton (1982) used to separate Messor from Aphaenogaster can be interpreted as occurring in the plesiotypic condition in Aphaenogaster. The relationships of the pheidoline genera clearly requires further study.

Aphaenogaster requires revision on a world-wide basis. Although about 270 specific and infraspecific names (including 11 names for fossil species) have been used in Aphaenogaster (see Appendix 1), only about 130 of these names represent valid extant species. Outside of North America, Smith (1961) revised the small group of New Guinea species, and Agosti and Collingwood (1987a,b) and Collingwood (1978) have presented recent keys to the European species. Cagniant (1968, 1982, 1984, 1987, 1988a,b,c, 1989a,b) has made numerous contributions to the taxonomy of the species of northern Africa.

Table 1 lists the names (along with original citations, information on types, and miscellaneous taxonomic notes) which I consider relevant to a discussion of the fulva-rudis-texana complex. The list includes flemingi and its synonym macrospina, both originally described as subspecies of texana, and huachucana, considered to be closely related to texana by Creighton

(1934, 1951). Although important in a broader phylogenetic treatment of the fulva-rudis-texana complex, flemingi and huachucana are morphologically distinct species, clearly separable from the sibling complex of interest to me by (among other characters) the presence of a distinct spatulate lobe at the base of the scape in the worker and queen.

The other names listed are pertinent to a discussion of the fulva-rudis-texana complex as a set of sibling species, or at least species which have proven difficult to delineate or identify using conventional morphological criteria. Included in the list is crinimera; in section 3.9 it is shown that Cole's (1953) placement of this form as a subspecies of huachucana is incorrect, and it is a probable synonym of texana. Also included is puncticeps, recently described from two workers collected in New Mexico; the status of this name is discussed in section 3.9.

The rest of the names were available at the time of Creighton's (1950) revisionary treatment of the Aphaenogaster in North America north of Mexico. The only previous revision of the forms in the fulva-rudis-texana complex was that of Emery (1895). Emery had recognized Roger's fulva as a good species and treated Buckley's Myrmica aquia as a subspecies of fulva. Emery described five new forms, all as varieties of fulva aquia; these were rudis, picea, azteca, pusilla, and texana. According to Creighton (1950), Emery's separation of forms was largely on the basis of the length of the propodeal spines. Wheeler (1915) raised texana to full species status and started the flood of seven descriptions of infraspecific forms of this species over a period of 24 years (see Table 1). Only two other forms in the complex were described between the appearances of the revisions of Emery and Creighton. Menozzi (1929) described silvestrii as a good species, and Enzmann (1947) described rubida as a variety of fulva. One other name requires comment: Myrmica opposita, described by Say (1836), was listed by Emery (1921) as a valid species of Aphaenogaster, but was not mentioned by Creighton (1950). Since the Say types appear to be lost, and the species (or even the genus) is unrecognizable from the

description, the placement of Myrmica opposita on the list of unrecognizable forms of Myrmicinae by Smith (1979) is likely to be permanent.

Creighton (1950) recognized four valid species in the fulva-rudis-texana complex (although he did not recognize the complex): fulva, rudis, texana, and miamiana. He also recognized two further subspecies: picea was treated as a subspecies of rudis, and carolinensis was treated as a subspecies of texana. The other available names were thrown in the taxonomic trash heap. Creighton synonymized both fulvescens and silvestrii with the typical texana, although he noted (p. 152) that "the variant silvestrii is known from so little material that its relationship to texana is doubtful." Enzmann's rubida was treated as a synonym of fulva, and the names aquia, nana, punctithorax, and pusilla were treated as nomen dubia.

Central to Creighton's treatment of the fulva-rudis-texana complex was his interpretation that the ant known to North American myrmecologists as fulva aquia was Emery's rudis, and his placement of aquia on the list of unrecognizable forms. Creighton stated (p. 147) that "the description [of aquia] is so poor that it offers no clue even as to the genus" and since no Buckley types are known to exist, Myrmica aquia will probably remain on the list of unrecognizable forms of Myrmicinae.

Creighton's explanation for treating nana, punctithorax, and pusilla as nomen dubia is worth quoting at length (p. 152): "After it was recognized that the head of texana is narrow, both in the worker and female, this fact was used as the basis for assigning to texana variants described from minors only. Since the minors of other species may also show this trait and since nearly all minors fail to show the definitive characters of their respective species, this practice is thoroughly deplorable. The naming of the forms nana Wheeler, punctithorax Cole and pusilla Emery has been wasted effort. Since the type series of all three forms consisted only of

minims, it will be impossible to determine what they actually represent. I propose, therefore, to discard the above three forms as impossible of exact recognition."

Creighton distinguished fulva workers from workers of other members of the fulva-rudis-texana complex by the size and shape of the mesonotal projection above the pronotum, and by the greater length and more vertical inclination of the propodeal spines. A comparatively wider head in miamiana, rudis, and picea was used to distinguish these forms from texana and carolinensis. He further separated miamiana from rudis and picea by its larger eye, slightly incurved propodeal spines, and very coarse, transverse rugules across the broad face of the propodeum.

The range of rudis (sensu stricto) was described by Creighton as southern New England west to Wyoming and south through the piedmont to Alabama. The form picea was considered to be a higher elevation subspecies, ranging from Nova Scotia south through New England and down the Appalachian Highlands to North Carolina. He considered rudis to be "slightly larger, lighter in colour and more heavily sculptured than picea" (p. 152), and separated the two forms in the key on sculptural differences. Creighton believed Emery's concept of aquia referred to an "intergrade" of rudis and picea.

The separation of texana (sensu stricto) and carolinensis in Creighton's key was based on the length of the large workers (5.5 mm versus 4.5 mm, respectively) and queens (7 mm versus 5.5 mm, respectively). The range of texana was given as "Arizona through Texas to the eastern Gulf and South Atlantic States", and carolinensis was considered to be distributed through the Piedmont and lower Appalachian levels from southwestern Virginia to northern Alabama. In the southeastern states, Creighton noted (p. 152) that "the typical texana ... is usually found in the coastal plain area." He also thought that there were areas in the southern Atlantic and eastern

Gulf states where intergrades formed, but there were areas in the Piedmont which had "pure stands" of carolinensis.

An earlier study by Talbot (1934) related ant distribution in the Chicago region to ecological factors and physiological tolerance to different conditions of temperature and relative humidity. She noted (p. 434) that "Aphaenogaster fulva aquia is rather common along road and railroad ditches or in open oak woods. Its variety, A. picea, occurs in beech-maple woods and especially along moist stream gullies of these woods." She hypothesized that the distributional differences between the two forms was correlated with their ability to withstand dryness. Two laboratory trials compared average survival times under conditions of low relative humidity at 30°C: 21 ants of each species were tested in each trial (unfortunately, it was not stated how many colonies were represented). As expected, survival times were significantly longer for the ants identified as fulva aquia. This work was not properly considered by Creighton (1950); clearly his treatment of rudis and picea as geographic subspecies was inadequate for explaining Talbot's observations.

Carroll (1975) presented a particularly insightful analysis of the fulva-rudis-texana complex as it pertained to the Florida fauna. Carroll's doctoral research consisted of an intensive study of the biology and ecology of the Aphaenogaster species found in Florida. The seven species of Aphaenogaster found in Florida that did not belong to the fulva-rudis-texana complex were morphologically distinctive and clearly reproductively isolated. Carroll could also recognize fulva with little difficulty (particularly the reproductives), although other workers had had problems in identifying this species in Florida (e.g., Van Pelt, 1958). Carroll discussed other forms in the fulva-rudis-texana complex as members of the "rudis/texana group", noting (p. 37) that "there does not seem to be, following current convention, a 'rudis group' distinct from a discrete 'texana group'."

Carroll concluded that no form in Florida corresponded to rudis, and that previous records of rudis and "fulva aquia" (e.g. Smith, 1930, 1965; Wheeler, 1932) were based upon misidentifications. Carroll (1975) also noted the extremely wide geographic range and diversity of habitats for texana as described by Creighton (1950, 1951), and questioned why Creighton had not considered the possibility that more than one subspecies or sibling species might exist under that name. Based on morphological comparisons with what seemed to be typical texana specimens from Brownsville, Carroll concluded that none of the Floridan Aphaenogaster could be assigned to that species.

Carroll noted that all "rudis/texana group" specimens south of Lake Okeechobee fit the description of miamiana by Wheeler (1932) quite closely. In north and central Florida, darker ants less typical of miamiana were generally found in wetter habitats, whereas ants in drier habitats had the lighter colour of the southern "miamiana" but were morphologically more similar to carolinensis. However, he could not reliably separate the two forms on the basis of morphology. In the absence of other evidence of reproductive isolation, Carroll was forced to treat all Florida representatives of the "rudis/texana group" under the single name carolinensis (with inferred synonyms miamiana, silvestrii, and nana). It was clear that he was uncomfortable with this treatment, however, as he maintained a distinction in his text between "typical" carolinensis and "miamiana-like" forms, marking the latter with an asterisk: "carolinensis*". It is interesting to note that in a subsequent paper, Carroll *et al.* (1981) identified a species of mycophagous Aphaenogaster as "miamiana sensu lato".

Recent regional studies on the Florida ant fauna have referred to the ants treated as carolinensis by Carroll (1975) under a diversity of names. In his study on the ant fauna of Alachua County, Johnson (1986) followed Carroll (1975) in using carolinensis for all "rudis/texana group" ants; he considered this interpretation consistent with his collecting

experience and examination of specimens. In their intensive analysis of the ant fauna of the Archbold Biological Station in Highlands County, Deyrup and Trager (1986) provisionally used the name texana, since Carroll's (1975) work had not been published and hence the elevation of carolinensis from the status of subspecies to species had not been formalized. The study of the ants of the Florida Keys by Deyrup *et al.* (1988) provisionally used the name miamiana. Deyrup *et al.* (1989) listed carolinensis, miamiana and texana as species "excluded from or dubiously included in" their preliminary list of the ants of Florida. Despite the enlightenment provided by Carroll (1975), it was obvious that the systematics of the representatives of the fulva-rudis-texana complex in Florida was not fully resolved. Carroll himself made this clear, concluding (p. 36) that "the genus Aphaenogaster is in dire need of taxonomic revision."

Outside of Florida, the taxonomic status of the fulva-rudis-texana complex Aphaenogaster has been considered on a regional basis in numerous local studies. Although the nature and quality of analysis varies widely in these studies, the best of them undoubtedly considered the taxonomic problems in some depth. Carter's (1962a,b) studies in North Carolina (with emphasis on the piedmont region) were intensive analyses relating ant distribution to plant communities. In the North Carolina piedmont, Carter (1962a) recognized fulva, "rudis rudis", and "texana carolinensis" in the fulva-rudis-texana complex. In bottomland forests rudis was considered to be a dominant ant and fulva a characteristic ant, while carolinensis was common in mature pine and oak-hickory forests. Carter (1962b, p. 163) noted that "many of the collections of Aphaenogaster from piedmont and coastal plain forests could not be determined with certainty as either A. texana or A. rudis." He further concluded that "confident separation of many North Carolina collections of A. texana into the races texana or carolinensis was impossible" using the key characters given by Creighton (1950). Although elevational differences were supposed to separate the ranges of A. rudis rudis and A. rudis picea in North Carolina, with picea occurring

higher in the mountains, Carter noted that "specimens have been collected in Craven and Carteret counties which apparently keyed to A. rudis picea". Carter (1962b, p. 163) concluded, "It would seem that the status of texana, carolinensis, rudis, and picea — ants which are extremely abundant in various forests — requires further study."

1.3. Crozier's Contributions

The contributions of the geneticist Ross H. Crozier were of critical importance both in recognizing the nature of the fulva-rudis-texana complex and showing a way to resolve some of the attendant systematic problems. Crozier (1975) detected three forms of 'rudis' based on chromosomal and electrophoretic evidence. He found two colour forms with distinctive karyotypes in the vicinity of Ithaca, New York: a dark brown form with an $n = 18$ karyotype and a light brown form with an $n = 16-18$ karyotype. The chromosome number variation in the light brown form seemed to be explainable by the presence of supernumerary chromosomes; when the light brown form had an $n = 18$ karyotype, it differed markedly in chromosome morphology from the karyotype of the dark brown form. He described both of these forms as occurring inland, with at least one of them ranging southwards along the Appalachian Mountains (he was almost certainly referring to the dark brown form). A third form with $n = 20$ was found from New Jersey south through the southeastern U.S.A.

Crozier (1977) presented an intensive analysis of the 'rudis' group in Georgia. He identified three distinct cytotypes, which he interpreted as sibling species: a lighter $n = 20$ "Coastal Plains" species, a darker $n = 22$ "Montane" species, and a further "Montane" species with $n = 18$. The two montane forms occurred sympatrically at Black Rock Mountain State Park; the $n = 18$ form was slightly darker than the $n = 22$ form and colonies were more frequent with increasing altitude. Near the "refreshment kiosk", there was marked microhabitat separation of the two forms, with the six colonies of the $n = 18$ form being found in logs and the seven colonies of the $n = 22$ form all nesting under stones. Crozier observed that the $n = 18$ karyotype was similar to the karyotype of the darker form of 'rudis' found near Ithaca, New York, but he noted (p. 33) that "a number of pericentric rearrangements have occurred between the two." The $n = 20$ karyotype was clearly the same as that reported in his earlier (1975)

paper. In Greene Co. (2.5 miles N Greensboro), a non-Robertsonian chromosome number polymorphism in the $n = 20$ form was detected, with some colonies having diploid numbers of 41 and 42.

Crozier (1977) also obtained the ($n = 18$) karyotype of fulva, which he considered very similar to the karyotype of the Montane $n = 18$ form. A colony from Alexander Springs, Florida, also had a similar $n = 18$ karyotype; this colony was identified as "rudis-group (?miamiana)". A distinctive $n = 22$ karyotype with a large metacentric was found for some colonies identified as dark 'rudis' from Alachua County, Florida. Karyotypes of Aphaenogaster lamellidens ($n = 19$) and A. treatae ($n = 21$) were also illustrated.

To seek evidence of gene flow between cytotypes, electrophoretic studies were performed utilizing four polymorphic loci (esterase, two malate dehydrogenase loci, and amylase). Although the karyotypes of fulva and $n = 18$ 'rudis' were considered similar, the two forms differed sharply in their amylase alleles. Esterase alleles gave some evidence of gene flow between $n = 20$ and $n = 22$ forms at one locality in Georgia, and also between fulva and sympatric 'rudis' populations, but Crozier concluded that such introgression must be extremely restricted, based on *Mdh-a* frequencies and the unambiguous chromosomal evidence. At Black Rock Mountain State Park, a single $n = 18$ colony taken at the lowest site (where $n = 22$ colonies were more abundant) had an *Amy*^{4/5} heterozygote queen; all other $n = 18$ colonies were fixed for *Amy*⁴ whereas all amylase alleles except *Amy*¹ were found in $n = 22$ colonies. There were statistically significant differences in allele frequencies between the $n = 18$ and $n = 22$ colonies collected near the "refreshment kiosk" for both *Mdh-a* and *Amy*.

A consistent colour difference between the $n = 18$ and $n = 22$ colonies (with the latter lighter) was evident to the eminent ant taxonomists William L. Brown, Jr. and Robert W. Taylor (Crozier, 1977), but the difference was considered to be too slight to be useful to separate or

recognize the taxa without the karyotype data. Crozier also noted that some of the $n = 18$ form workers showed weak development of the mesonotal projection often considered diagnostic of fulva. No other morphological evidence of species differentiation was found; the recognition of the different sibling cytospecies depended on karyotypes and was supported by electrophoretic data. Crozier (1977, p. 17) concluded that "the 'rudis' cytotypes in Georgia are sibling species whose close morphological resemblance may reflect ecological but not genetic similarity."

1.4. Karyotyping in Ants

With more than 500 species karyotyped (Imai *et al.*, 1988a), the ants are one of the cytologically best studied families of Hymenoptera. Among the people who have published on ant cytogenetics, R. H. Crozier and H. T. Imai stand out for their contributions to the development of karyotyping technique, to the accumulation of a large cytological data base, and to the development of theoretical models of karyotype evolution.

Their earlier methodology (Imai, 1966; Crozier, 1968) evolved into an air-drying technique with Giemsa staining (Imai *et al.*, 1977, 1988a; Crozier *et al.*, 1986); this technique gives bands that are interpreted as C-bands although no barium hydroxide treatment is used. Other authors have proposed modified versions of their methods. Mehlhop and Gardner (1982) suggested that their flame-drying technique was useful under field conditions since it was fast and could be used under humid conditions. Cokendolpher and Brown (1985) suggested that their air-drying method could be used for a variety of arthropods and could be performed in the field. Palomeque *et al.* (1987) stained for both C-bands and nucleolar organizing regions. Their C-banding procedure followed Sumner (1972) in utilizing barium hydroxide.

Karyotyping has proved to be of value in ongoing research to resolve sibling species complexes in *Myrmecia* (species near "*pilosula*"; Crosland *et al.*, 1988; Imai *et al.*, 1988b) and the *Leptothorax* "*muscorum*" complex (Loiselle *et al.*, 1990). The importance of understanding the mechanisms of chromosomal reorganisation in karyotypically polymorphic species has been demonstrated, for example, by Crozier (1970) and Imai and Taylor (1989).

Chromosome numbers from $2n = 2$ to $2n = 84$ ($n = 1$ to 42) have been found in the ant genus *Myrmecia*, a range which exceeds that known for any other eukaryote genus; all other ants have diploid numbers comfortably within this range, except for *Nothomyrmecia macrops* with $2n = 94$ and *Platythyrea tricuspidata* with $2n = 92-94$ (Imai *et al.*, 1990). Haploid

karyotype numbers reported for Aphaenogaster range from 11 to 23. In addition to Crozier's data (section 1.3), the following haploid numbers have been found in Aphaenogaster: subterranea $n = 11$ (Switzerland; Hauschteck, 1962); smythiesi $n = 11$, osimensis $n = 16$, famelica $n = 17$ (Japan; Imai, 1971); tipuna $n = 17$ (Taiwan; Hung et al., 1972); gibbosa $n = 11$ (Switzerland; Hauschteck-Jungen and Jungen, 1983); depilis $n = 17$, sardoa $n = 17$, testaceopilosa $n = 17$ (Mediterranean; Hauschteck-Jungen and Jungen, 1983); beccarii $n = 15$ (Malaysia; Imai et al., 1984a); beccarii $n = 23$, smythiesi $n = 17$ (India; Imai et al., 1984b). A Robertsonian polymorphism with $2n = 45, 46$ was found in longiceps (Imai et al., 1977). The discrepancies in chromosome number for beccarii between Malaysia and India, and for smythiesi between Japan and India, may suggest that more than one species resides under each name.

Imai et al. (1988a) presented the most recent summary of karyotype evolution in ants, further developing and refining the hypotheses of Imai et al. (1977, 1984b, 1986). Spontaneous chromosomal mutations observed in ant karyotypes were placed into eight classes: Robertsonian rearrangements, translocations, pericentric inversions, size variation of heterochromatic short arms by tandem growth of constitutive heterochromatin with secondary elimination of constitutive heterochromatin, partial deletion or duplication, supernumerary (B-) chromosome variation, trisomy or monosomy, and polyploidy. Of these, Robertsonian rearrangements, translocations, and pericentric inversions were considered to have the greatest significance in the evolution of the observable karyotype differences in ants (it should be noted that paracentric inversions generally are not detectable in ant karyotypes with the existing methodology).

Imai et al. (1977) made some overall conclusions on the direction of chromosome number evolution in ants that were essentially supported by Imai et al. (1984b, 1988a). There was no support for the "fusion hypothesis", in which the ancestral ant karyotype had a high chromosome number, and most changes in chromosome numbers were due to fusions; this was the general

model of karyotype evolution in animals supported by White (1973). Instead, they adopted a "modal hypothesis", which they considered as representing a subset of the "fission hypothesis". The fission hypothesis stated that the ancestral ant karyotype had a very low chromosome number, and the primary direction of chromosomal number change was to increase through fissions. The modal hypothesis stated that the ancestral ant karyotype had a chromosome number close to the present day mode of $n = 11$, and both fissions and fusions had led to chromosome number divergence from the modal value. However, to maintain the modal value, fissions must have been much more common than fusions, since the chromosome number distribution has a substantial positive skew. To account for the present-day distribution of chromosome numbers under the modal hypothesis, they estimated that the fission to fusion ratio must have been about 10:1.

A finding of great significance was the nonrandom distribution of Robertsonian polymorphisms and translocations, originally noted in large cytological studies of both Australian ants (Imai *et al.*, 1977) and Indian ants (Imai *et al.*, 1984b). Robertsonian polymorphisms occurred primarily in species with higher chromosome numbers ($n > 12$) while translocation heterozygotes were found mainly in lower-numbered species of $n \leq 12$ (Imai *et al.*, 1988a). Lineages with higher chromosome numbers tended to show substantial variation in chromosome numbers, whereas lineages with lower chromosome numbers tended to show much less variation in chromosome number, even though there was often substantial karyotype restructuring (Imai *et al.*, 1977).

Two hypotheses were offered to explain this pattern. Imai *et al.* (1977) first suggested that translocations might occur at a relatively low but constant rate in all lineages, but in higher numbered lineages, Robertsonian changes (primarily fissions) obscure the evidence of translocations that are observable in lower numbered lineages in the form of observable

heterozygosities. The currently favoured "minimum interaction hypothesis" was developed in detail by Imai *et al.* (1986, 1988a) to account for karyotype evolution in ants and possibly other animals. Spontaneous chromosomal mutations are expected to occur most frequently when the chromosome number is small (since the individual chromosomes will, on average, be large), and the genetic risks of mutations, particularly reciprocal translocations, can be reduced by increasing the chromosome number and/or arm number by centric fission and pericentric inversion. Pericentric inversions will tend to move the centromere towards the centre of the chromosome. After a fission, a telocentric chromosome must grow a short arm through the tandem growth of constitutive heterochromatin before it can undergo pericentric inversion; this should occur rapidly because of telomeric instability following a fission. A "fission-inversion cycle" will operate to change chromosomes with two euchromatic arms (metacentric, submetacentric, or subtelocentric) to two single-armed (telocentric) chromosomes. After the single-armed chromosomes grow heterochromatic short arms, they can be converted to chromosomes with two euchromatic arms, and hence are susceptible to centric fission.

The tandem growth of constitutive heterochromatin can be quite rapid, to the point that the heterochromatic "short arm" can even exceed the length of the euchromatic "long arm" in ant chromosomes. Imai *et al.* (1988a) called such chromosomes "pseudoacrocentrics". Because heterochromatin tends to form nonspecific associations, translocation risks will increase as the heterochromatic arm grows. Centric fusion with the loss of heterochromatin provides a way of temporarily avoiding this problem, but now fission will be favoured to produce two telocentric chromosomes. Thus, the "fission-fusion cycle" was considered to be only a short-term solution in karyotype evolution, with the "fission-inversion cycle" likely to predominate.

The minimum interaction hypothesis is of critical importance because it gives a directionality to how karyotype evolution is expected to proceed. Hence, if two species have

karyotypes which differ by only a single fission or fusion event, we would expect it to be much more likely that it was a fission, unless other evidence contradicted this. Given the chromosome number range known in Aphaenogaster, we would expect that Robertsonian polymorphisms have been much more important than translocations in the evolution of the karyotype in this genus, and under the minimum interaction hypothesis, the trend has been to evolve towards a higher chromosome number. In section 3.2, these predictions are tested with the karyological data obtained during the research for this thesis.

1.5. Electrophoresis in Ant Systematics

Crozier (1981, p. 357) noted that "allozyme differences between closely related ant species are generally slight, and most genetically characterized sibling species have been discovered on the basis of chromosomal differences". Nonetheless, electrophoresis of isozymes has been used to recognize sibling species in, for example, the Australian Iridomyrmex near purpureus (Halliday, 1975, 1979, 1981), the Rhytidoponera impressa group (Ward, 1980a), and the Leptothorax "muscorum" group (Heinze and Buschinger, 1988; Heinze, 1989), in addition to Crozier's results in Aphaenogaster (section 1.3). Pearson and Child (1980) used differential allelic frequencies in an esterase polymorphism to provide evidence that the microgyne form of queen found in nests of Myrmica rubra was probably a separate, socially parasitic, species (but see Bourke and Franks, 1991, for an alternative interpretation).

The fire ants (genus Solenopsis) of the southern United States have been subjected to extensive electrophoretic analysis in order to study their systematics, social structure, and other aspects of population genetics. The majority of this work has come from the laboratory of Ken Ross at the University of Georgia; key findings include the demonstration of introgression between the imported fire ants Solenopsis richteri and S. invicta (Ross et al., 1987a) and the verification of the monogyne and polygyne forms of S. invicta as conspecifics (Ross et al., 1987b).

Hung and Vinson (1977) and Hung (1985) obtained unusual results in electrophoretic analyses of a hybrid population of male S. geminata hybridized with queen S. xyloni. For malate dehydrogenase, there was repression of the paternal alleles in alate females but not in workers. For another eight enzymes examined only in the worker caste, six exhibited repression of the paternal alleles and one (amylase) was clearly hybrid in nature. The esterase banding pattern was essentially hybrid in nature, but one maternal band was missing and another band was present

that did not appear to occur in either parent species. Electrophoresis has also been used to verify natural hybridization between Lasius niger and L. alienus (Pearson, 1983b) and laboratory hybridization in cross-breeding experiments involving five species of Epimyrma (Jessen and Klinkicht, 1990).

Electrophoretic investigations of ants have used a wide variety of methods and materials. The laboratory of Ken Ross has tended to use horizontal starch gel electrophoresis on the alitrunks and gasters of queens (Ross *et al.*, 1985, 1987a,b), while Ward (1980a) used whole worker ants and polyacrylamide disc gel electrophoresis. Heinze and Buschinger (1988) established "genetic fingerprints" for several leptothoracine species using isoelectric focusing of esterases, but reliable results could only be obtained with unpigmented pupae. When Heinze (1989) made a more intensive study of the Leptothorax "muscorum" group in North America, he switched to using cellulose acetate plates and adult workers. When they are available, males can be very useful in helping to determine the genetic system of inheritance of an allozyme, since they are haploid.

Sampling plans have been varied, in large part due to different social structures of colonies but also due to different goals of investigators. Crozier (1977) sampled at least eight worker ants per colony, so that the probability of misclassifying a heterozygote queen as a homozygote was small (≤ 0.0078) for a given allozyme. Crozier (1974) found that electrophoretic evidence was consistent with the hypothesis that Aphaenogaster 'rudis' queens mated with a single male, thus only three nonduplicated genomes are present in the entire colony. Any single daughter (worker or gyne) contains two nonduplicated genomes, but all daughters contain their father's single genome. For a colony with one singly-mated queen, it is easy to show that the expected proportion of nonduplicated genetic material contained within N randomly sampled workers (or gynes) is given by $1 - (1/2)^{N-1} / 3$. Thus there is a rapidly diminishing rate

of return for sampling additional workers in a colony when screening for genetic variation in a population. For this reason, Ross *et al.* (1987b) scored only one queen per colony. Ward (1980a) sampled six workers per nest, but he estimated that this accounted for about six nonduplicated genomes, since the mean number of inseminated reproductive females per nest was about three to four and there was a certain degree of relatedness among the reproductive females.

For the few cases where electrophoretic data on ants have been used for phylogenetic inference, the analysis has essentially been phenetic. The technique of clustering on Nei's genetic distance values as a method for estimating phylogeny was thoroughly criticized by Carpenter (1990). Heinze and Buschinger (1988) used similarity of esterase "fingerprints" to test Emery's rule (Hölldobler and Wilson, 1990), but again the inference is essentially phenetic. As for any other set of characters, the use of electrophoretic data for inferring phylogeny must be analyzed with a fundamentally sound methodology (Carpenter, 1990).

1.6. Rationale for a Morphometric Approach

When individuals can be reliably identified to group on the basis of cryptic characters (e.g., genetic markers, genitalia), these samples can be used to search for external morphological characters that can be used to classify individuals for which the cryptic characters are either unavailable or available at greater cost. When several measurement characters are utilized, the multivariate statistical methodologies of discriminant analysis and canonical variate analysis are powerful ways of utilizing and summarizing the data. Discriminant analysis constructs functions which, based on some criterion (e.g., overall misclassification rate, costs of misclassification) minimize classification errors. By using a variable selection procedure, such as stepwise selection, weak or redundant variables can be eliminated from the discriminant model, and some idea of the relative value of the useful variables in the model can be adduced. Canonical variate analysis provides a powerful method of reducing the dimensionality of the data by constructing new canonical variables, uncorrelated with one another, that are linear functions of the original variables.

The theory and application of discriminant analysis were reviewed by Lachenbruch (1975, 1982). The most commonly used method of discriminant analysis is linear discriminant analysis, which assumes that the populations have multivariate normal densities and a common covariance matrix. Fisher (1936) derived the linear discriminant function for the case of two groups, and Rao (1948) extended this for the case of more than two groups. Blackith and Reyment (1971) presented the theory and application of canonical variate analysis. Lubischew (1962) gave an excellent analysis on how the choice of characters affects discriminant ability, using examples from sibling species complexes in the flea beetle genera Haltica and Chaetocnema (Chrysomelidae: Halticinae). Baker et al. (1972) used both linear discriminant analysis and

canonical variate analysis to examine the degree of morphological differentiation among three chromosomal races (treated as subspecies) of the bat Uroderma bilobatum.

Multivariate statistical methodologies have not been used much in ant systematics, although several studies (e.g., Wing, 1968; Francoeur, 1973; Ward, 1985, 1989a) have made extensive use of measurement data and scatterplots of pairs of variables (including ratio variables). Reyes Lopez and Porras Castillo (1984) used linear discriminant analysis on ten forewing measurements to obtain perfect separation of 111 queens from five nests (52 specimens) of Goniomma hispanicum and four nests (59 specimens) of G. baeticum, but it is never clear in their analysis how reducing the number of variables used might affect the classification results. Furthermore, the small number of colonies relative to the number of variables and the lack of information on the geographical distribution of the sampled colonies requires one to question the general applicability of their analysis. Elmes (1978) used canonical variate analysis on 12 variables to get excellent morphological separation of the males and queens of three species of Myrmica: scabrinodis, sabuleti, and hirsuta, the latter identified as a new species of workerless parasite on sabuleti. His keys were based entirely on canonical variate scores, and have the unusual feature of giving a confidence level for correct classification.

A methodologically strong paper that used linear discriminant analysis in the classification of social insects (honey bees, alas, not ants) was that of Daly and Balling (1978). They measured 25 morphometric characters on 101 samples of Africanized honey bees and 297 collections of European honey bees collected throughout the Western Hemisphere. Usually 10 bees were measured per sample; when the sample means for all 25 variables were utilized in the discriminant analysis, there was no misclassification of samples; four of the variables could be eliminated without any misclassification. Various subsets of characters, chosen for maximizing information while minimizing the costs of measurement, gave observed misclassification rates as

high as 4.8% for a two-variable subset. When all bees were treated as individuals, the misclassification rates were substantially higher, with observed rates ranging from 4.4% when all 25 variables were used to 11.5% for the two-variable subset.

Multivariate morphometric analyses can be used for sophisticated analyses of evolutionary pattern (e.g., Bookstein *et al.*, 1985; Rohlf and Bookstein, 1990). In this study, the emphasis has been on the delineation and recognition of the component forms in the fulva-rudis-texana complex. Hence, discriminant analysis and canonical variate analysis have been chosen as the appropriate multivariate methods for what is essentially a taxonomic problem. These analyses have been supplemented by numerous two-variable scatterplots as aids to understanding the nature of interspecific and intraspecific variation in this complex.

2. METHODS

2.1. Field Collection of Colonies and Laboratory Maintenance

I collected most of the live colonies utilized in this study in 1987–1989. Other valuable colonies were received from J. Carroll, S. Cover, J. Cumming, L. Ling, S. Porter, and J. Trager. A summary of the localities for which one or more colonies were karyotyped is presented in Table 2. The localities in eastern North America (all localities except those from Arizona and Texas) are plotted on Map 1. In addition to colonies of the fulva-rudis-texana complex, colonies of other species of Aphaenogaster (lamellidens, tennesseensis, and treatae) were collected when available for outgroup comparisons.

The limiting factor in this study was the time required to process the material collected; it was certainly not the availability of the ants themselves. Emphasis was placed on collecting over a broad geographical range (Map 1), and hence the number of colonies sampled at any one site had to be kept small. Ideally, I tried to collect four colonies of each form for certain localities, so that intercolony variation within localities could be examined; this was most important for the electrophoretic work but was also important for the chromosomal and morphological studies. However, the number of colonies sampled at a particular site was highly dependent on the logistics of covering large distances under tight time constraints, the diversity of nesting microhabitats and differentiable forms present at a particular locality, and the degree of confidence I had in my field identifications. The overriding consideration was to attempt to maximize information for the time and resources available.

The choice of collecting sites was guided by general ecological and biogeographical considerations, as well as the range summaries for the recognized taxa given by Creighton (1950) and Smith (1979). Numerous local studies were consulted for additional hints; the studies of Carter (1962a,b) and Carroll (1975) were particularly useful. A special effort was made to

collect at two stations in Georgia sampled by Crozier (1977); these were GA1, where Crozier had noted a non-Robertsonian chromosome number polymorphism in the $n = 20$ form, and GA3, where Crozier had found $n = 18$ and $n = 22$ forms in sympatry with microhabitat differentiation. Although the collections of 1987 produced relatively little data, they were invaluable in directing collecting efforts in 1988–1989.

Aphaenogaster colonies of the fulva-rudis-texana complex usually nest in rotten wood, in leaf litter, and in soil with or without a covering object; the rotten wood and "under stone" microhabitats are the easiest to sample from. When a colony was broken into, as much of the colony as possible was scooped into a Ziploc freezer bag or polystyrene container (some colonies were adept at chewing through freezer bags if not kept sufficiently cool). Special attention was paid to collecting as much brood as possible. Although it was always considered desirable to collect the nest queen, it was not usually a serious problem if she was not obtained. Queenless colonies maintained good colony cohesion, and the workers would rear any existing brood (with some young female larvae reared as gynes) and lay haploid (male-producing) eggs. Imai and Yosida (1965a) found in the Japanese species Aphaenogaster osimensis that males produced from worker-laid eggs were morphologically normal and had a normal chromosome number in both somatic and germ cells.

The primary sample of workers and available alates for morphological studies was usually preserved (in 85% ethanol) at this time; sometimes this was left to a future date in the laboratory if, for example, the colony was small or field time was very limited. A special "Aphaenogaster" colony number was assigned (A001 to A446 were used in this study) and field notes were taken. The live colonies were usually stored in coolers with ice to prevent overheating in the car.

Back in the laboratory, the ants were extracted from any debris, usually with a technique modified from Carroll (1975). The debris with the colony was spread out in a pan ringed with

Fluon; as the debris dried out, the ants would move into a plastic vial with a hole in the lid and damp paper inside. The ants could then be efficiently transferred to an artificial nest.

In the laboratory, colonies were maintained in artificial nests to rear brood for karyotyping, to rear alates, and sometimes to try to rear more workers for electrophoresis. Artificial nests were made out of clear polystyrene containers; three sizes were used (approximate dimensions of L $\times$ W $\times$ H in cm: 12 $\times$ 8½ $\times$ 6, 17 $\times$ 12 $\times$ 6, 13½ $\times$ 12½ $\times$ 3½). To maintain sufficient humidity, a layer of plaster of Paris was poured into the bottom of each container and wetted occasionally. Within a container, a nesting site was provided: this consisted of a rectangular block of 6 mm thick transparent red plastic raised above the surface of the plaster by a bead of clear silicone attached to the plastic. These "nesting blocks" provided easy access to the colony for harvesting karyotypable brood and reared alates, particularly if the container was cooled for a few minutes. The ants were supplied with water and fed the artificial diet of Bhatkar and Whitcomb (1970) and pieces of various fresh or frozen insects (primarily mealworms and frozen American cockroaches).

2.2. Chromosomal Studies

Imai (1966) related stage of development of immature ants to the relative frequency of cell divisions in the preferred tissues (brain, testes, ovary) for karyotyping. The tissue I preferred to use for karyotyping ants was the brain of the prepupa of any caste, since this gave excellent mitotic metaphase chromosomes. Testes of the pupal male were also used; the chromosome morphology was usually not as clear as in spreads made from brains, but there was usually considerably more spreads available for counting and they were critical for examining differential supernumerary chromosome variation between germ and somatic cell lines. Imai (1966) suggested that the optimal stage of development for karyotyping testes was when the eyes of the male pupa were "slight rouge", but in Aphaenogaster the eyes should be darker than this. No results in this thesis are based on karyotyping ovaries. Since males are haploid, their karyotypes are simpler, easier to count and no matching of chromosomes is necessary. However, diploid karyotypes allow one to detect chromosomal heterozygosities.

Eggs were used to karyotype two colonies, following Angus (1982, 1983) and a suggestion from Dave Maddison (pers. comm.). Aphaenogaster treatae colonies were collected without brood from Pinery Provincial Park, Ont., and this species did not survive well in the laboratory; other colonies collected in the US with brood did not survive long enough to karyotype. Using eggs was thus a last chance attempt to karyotype this species in order to confirm the results of Crozier (1977).

Most slides of karyotypes were prepared with the flame-drying technique of Mehlhop and Gardner (1982). The air-drying techniques of Crozier (1970), Imai *et al.* (1977), and Cokendolpher and Brown (1985) were tried briefly with varying degrees of success, but I quickly settled on the flame-drying technique since it was fast, reliable, and gave some excellent preparations. Mehlhop and Gardner's procedure was modified as follows.

1. Slides were soaked in 70% acid alcohol for at least 24 hours, as in Mehlhop and Gardner (1982). They were then carefully washed with soap and water, thoroughly rinsed with a sequence of running tap water, distilled water, and ethanol, and then stored in absolute alcohol.
2. The optimal time for the dissected tissue to sit in the hypotonic solution (0.8% sodium citrate, no colchicine) appeared to be about 25 minutes; this time was used for most preparations.
3. Slides were prepared in batches of 4–6 slides, with the time of each preparation starting its hypotonic treatment recorded. The three fixative solutions were prepared fresh for each batch of slides, just prior to the first preparation being ready to come out of the hypotonic solution. Fixatives were prepared in 60 ml eye dropper bottles, in the following quantities: fixative I, 6 ml absolute methanol, 6 ml glacial acetic acid, 8 ml distilled water; fixative II, 7 ml absolute methanol, 7 ml glacial acetic acid; fixative III, 9 ml absolute methanol, 3 ml glacial acetic acid.
4. Slides were removed from the absolute ethanol, flamed, and allowed to cool just prior to the end of the hypotonic treatment for the first preparation.
5. Tissues were moved using a scoop made from a fine piece of teflon tubing. Other ant cytotaxonomists have used pasteur pipettes (Imai, 1966; Imai *et al.*, 1988) or insect pins or dissecting needles (Mehlhop and Gardner, 1982), but I tended to lose the tissue too frequently with these techniques. The teflon scoop makes it much easier to move testes relatively intact; the separate follicles fall apart very easily when moved with other instruments.

6. As soon as the tissue is moved from the hypotonic solution to the clean slide, one drop of fixative I was placed directly on the tissue before it was inclined and washed with 8–15 drops of fixative I; this helps to "stick" the tissue to the slide.
7. The two drops of fixative II should be placed on each side of the centre of the slide, so that as they spread they run together.

After drying for at least one day, slides were stained in 4% Giemsa (Burr's improved R-66, BDH Chemicals) in Sorensen's phosphate buffer, pH 6.8, for 10–25 minutes. Three different bottles of Giemsa solution were used during this study, and the staining ability of the Giemsa seemed to vary among bottles and with the age of the concentrated solution. The slides were stained in Coplin jars; at the end of staining, the jars were gently flooded with distilled water to float off the metallic scum formed on the surface when Giemsa is exposed to air. Individual slides were then given a brief rinse in distilled water and allowed to dry thoroughly.

Slides were examined using a Zeiss Photomicroscope II belonging to the Carleton-Ottawa Institute of Microscopy. Temporary cover slips were mounted with a drop of immersion oil prior to examination. Although Imai *et al.* (1977) noted that the slides can be examined without using a coverslip, resolution with the particular microscope used in my research seemed to be improved (and the objective kept cleaner) by using a coverslip. All chromosome counts and photographs were made using a Zeiss 63× planapo objective with a numerical aperture of 1.4.

Initially I tried to get at least 10 clear, consistent chromosome counts spread over at least three slides for each colony. After the first year of gathering data, this criterion was judged overly stringent; only rarely was anything interesting found on a subsequent slide when the first slide had a standard karyotype. Hence it was decided to examine only one slide per colony unless an interesting karyotype was found, or there was some doubt on the identity of the form

to which the colony belonged. This allowed a larger number of colonies to be screened than would have been possible under the original sampling plan; furthermore, there was generally sufficient replication from other colonies collected from both the same and different localities to establish the karyotypic identity of each colony unequivocally. Additional slides were prepared and examined if possible whenever unusual results were obtained.

Selected karyotypes were photographed using Kodak Technical Pan film and developed with Kodak HC-110. The representative karyotypes in Figures 1 – 3 were prepared from selected prints; to some degree the matching of homologous chromosomes in diploid karyotypes was arbitrary and thus there are likely some mismatches. However, any mismatches would be among very similar chromosomes and would not unduly affect the overall impression of the karyotype.

Measurement data for selected karyotypes were obtained using the digitizing system in the laboratory of James Bogart, University of Guelph. The photographic negatives were projected on the screen of a Bell and Howell microfiche reader and a digitizing pen was used to trace the image of each chromosome arm. The microcomputer chromosome analysis package CHROMPAC received the digitizer coordinate data directly for subsequent analysis. The digitizing equipment and CHROMPAC were more fully described by Bogart and Tandy (1981) and Green *et al.* (1980).

CHROMPAC provides measurements in absolute value and as a proportion of total genome size for the average short arm, long arm, and total length for each chromosome. For diploid karyotypes, a "haploidized" karyotype is obtained by averaging the pairs of chromosomes considered to be homologues by the operator. Centromeric ratio ($r = \text{length of long arm} / \text{length of short arm}$) and centromeric index ($i = \text{length of short arm} / \text{total length}$) are calculated for each chromosome. Based on the relative arm lengths, each chromosome is classified (abbreviation and critical centromeric index values in brackets) as telocentric (t, $i = 0-0.125$),

subtelocentric (st, $i = 0.125 - 0.250$), submetacentric (sm, $i = 0.250 - 0.375$), or metacentric (m, $i = 0.375 - 0.500$). The classification system used (Green *et al.*, 1980) is modified from that of Levan *et al.* (1964).

The chromosome nomenclature used by Crozier and Imai (e.g., Imai *et al.*, 1977, 1988a) is also based on Levan *et al.* (1964), but differs in some details. Crozier and Imai define metacentric, submetacentric, and subtelocentric similar to Green *et al.* (1980) but abbreviate the terms with upper case (M, SM, ST) rather than lower case letters. They further break down the telocentric category of Green *et al.* (1980) into acrocentrics (A) and telocentrics (T), with telocentrics having the smaller short arm. Imai *et al.* (1977) pointed out that the differentiation of M, SM, and ST chromosomes is useful for describing a karyotype, but the three classes do not reflect any fundamental difference in chromosome organization: they all have two euchromatic arms. Imai *et al.* (1988a) referred to "metacentrics in the broad sense" to refer to M, SM, and ST chromosomes as a group, which they abbreviated $\bar{M}$. Based on an analysis of 16,817 chromosomes obtained from 723 mammalian species, Imai (1975, 1976) showed that the centromere in mammalian chromosomes was nonrandomly distributed. Expressed in terms of the weight of the short arm as a percentage of the haploid genome (S_w), telocentric chromosomes had $S_w < 0.1$, acrocentric chromosomes had $0.1 \leq S_w \leq 0.6$, and the rest (M, SM, ST) had $S_w > 0.6$. The critical S_w values have not yet been worked out in ants, but the three main categories ($\bar{M}$, A, T) were judged intuitively (Imai *et al.*, 1977). Imai *et al.* (1977) further differentiated "pseudoacrocentric" chromosomes (abbreviated A^M) which might appear to be M, SM, or ST chromosomes on the basis of the centromeric index, but had one euchromatic arm and one unusually long heterochromatic arm. Definite recognition of pseudoacrocentric chromosomes requires C-banding, which differentially stains heterochromatin dark.

The chromosome classification systems of both Green *et al.* (1980) and Imai *et al.* (1977, 1988a) are used in this thesis. The CHROMPAC classification (which is strictly quantitative) is modified where I believe it is appropriate to better conform to the nomenclature of Imai *et al.* (1977, 1988a), as further discussed in section 3.2. Although a specific C-banding protocol was not used in my research, bands similar to those obtained by Imai *et al.* (1977) were observed in some preparations. I believe that in most cases a reasonable estimate can be made as to which arms are entirely heterochromatic, and a more accurate picture for analyzing karyotype evolution in *Aphaenogaster* can be obtained with Imai *et al.*'s (1977, 1988a) modified nomenclature. The classification system can easily be determined, where necessary, by the case of the abbreviations: lower case for CHROMPAC, and upper case for the Crozier and Imai system.

2.3. Electrophoresis

The electrophoretic investigations conducted during my thesis research were motivated by the results obtained by karyotyping and impressions gained from morphological and field studies. Based on these results, the primary purpose for electrophoresis was to obtain unambiguous evidence of species differentiation between two forms with very similar $n = 22$ karyotypes, and confirmation of the apparent hybrid nature of colony A221. Of course, further evidence of the degree of reproductive isolation between pairs of forms was considered highly desirable, but not essential at the current stage of my research.

An initial screening for potentially useful isozymes using horizontal starch gel electrophoresis was performed at Cornell University by the Cornell Laboratory for Ecological and Evolutionary Genetics (CLEEG) under the direction of Bernie May. (A very useful training session was provided with the screening.) Ten colonies were screened for 48 enzyme systems on four buffer systems, using horizontal starch gel electrophoresis. The results of the screening suggested that 29 enzyme systems were worth further investigation (Table 4). The CLEEG methodology is described in the manuals by May (1980) and May *et al.* (1988).

Further electrophoretic investigations were conducted in the laboratory of George Carmody at Carleton University using the CLEEG methodology. The 14% w/v starch gels were prepared using equal parts of hydrolyzed potato starch supplied by Sigma and Connaught Laboratories. Gels were cooled during running by metal pans filled with crushed ice. Although the initial screening at CLEEG had used homogenates of eight whole worker ants ground together, individual ants (workers except as noted) were usually used in the investigations at Carleton. Staining recipes followed were essentially those of May *et al.* (1988), but manuals by Aebersold *et al.* (1987) and Cheliak and Pitel (1984) also provided useful suggestions and slight modifications. Further details on the enzymes run and sample sizes are given in section 3.4.

2.4. Morphological Studies, Including Morphometrics

Samples of specimens from a colony were collected into 85% ethanol and designated as either a primary or secondary sample (there could be more than one secondary sample, but only one primary sample). By preference, the primary sample was obtained at the time the colony was first collected, but occasionally (as noted in section 2.1) this sample was collected in the laboratory. One or more secondary samples were often collected from the colony in the laboratory; these samples could include reared reproductives, nest queens, unusually small or large workers, and abnormal individuals. An advantage of long laboratory rearing is that minor workers will often be produced although none were present when the colony was first collected; these small workers (usually the only size class found in incipient colonies) have frequently been the cause of taxonomic confusion (see section 1.2). Any sample not preserved on the date the colony was first collected was also labelled with the date of collection in the laboratory.

Workers in the primary sample were laid out in rows and six specimens were randomly selected using random number tables (such as the fine tables found in Raktoe and Hubert, 1979). The six specimens were mounted individually and were labelled with a label denoting their position in the random sample. Obviously this was not a truly random sample from all workers present in the colony since the primary sample itself could not be obtained randomly. However, I am inclined to believe that the greater source of bias comes in the laboratory when the specimens are selected for measurement, and hence the extra trouble to randomize the selection from the primary sample was worthwhile.

Usually two further pins of workers were mounted, with three workers on a pin, selected from the remainder of the sample in the usual myrmecological manner (a small, medium, and large worker). A selection of male and queen ants were also mounted (usually three of each). Under normal air drying, various sclerites of the males tend to collapse. Superior male

specimens were prepared from selected colonies by John Swann using the critical point dryer at the University of Guelph. Extra specimens, including any anomalous ants, were mounted if desired. Studies of morphological differentiation among workers of the different genetically delineated forms was greatly aided by the preparation of representative SEM photographs of the (1) head, (2) alitrunk, and (3) petiole plus postpetiole by Lewis Ling of Carleton University. Representative male genitalia were also prepared by soaking them in 10% KOH for three hours at room temperature, rinsing them thoroughly with distilled water, and storing them in individual genital capsules in a drop of glycerin.

The morphometric analysis in this thesis is based on twelve measurements taken on the first randomly selected worker for each colony utilized. The additional material mounted was used to make qualitative assessments of morphological differentiation, and is intended to serve as a base for further research.

All measurements used in the morphometric analysis were made at $50\times$ magnification using a Nikon SMZ2T zoom dissecting microscope fitted with an ocular scale in the laboratory of Steve Marshall at the University of Guelph. The scale was calibrated and checked frequently for accuracy with an ocular micrometer. (For specimens illustrated in Figures 10–13 that were not used in the morphometric analysis, scale measurements were made on my own Leitz dissecting microscope at $63\times$ magnification.) The twelve measurements made are listed as follows.

HW Head width: with head in full-face view, measured directly above the eyes.

HL Head length: maximum length down the centre of the head from the top of the head to the top of the clypeal notch. This measurement differs slightly from the HL of most other ant taxonomists.

- SL** Scape length: maximum linear measurement of the length of the antennal scape (first antennal segment), excluding the basal condyle.
- EL** Eye length: maximum length (diameter) of eye.
- WLA** Weber's length of alitrunk: similar to the WL of Ward (1980b), which was defined as a diagonal measurement "from the anterior margin of the pronotum (excluding the collar) to the posterior extremity of the metapleural lobe". The measurement was taken with the specimen in approximate lateral view, but slightly tilted so that both reference points were sharply focused.
- MH** Mesonotal height: maximum measurement from the promesonotal suture to the posteroventral corner of the mesonotum.
- SPL** Spinal length: length of the propodeal spine, measured from the centre of the propodeal spiracle to the tip of the spine. The left spine was measured unless broken, in which case the right spine was used.
- ISPL** Interspinal length: distance from the centre of a straight line extending between the tips of the propodeal spines and the centre between the bases of the spines. The specimen was oriented to maximize this measurement. If one of the spines was broken, its length was estimated by the other spine.
- SPD** Spinal distance: the distance between the tips of the propodeal spines.
- FW** Femur width: maximum width of the hind femur.
- FL** Femur length: maximum length of the hind femur plus trochanter. This is an easier and more precise measurement than measuring the femur only.
- TL** Tibia length: maximum length of the hind tibia.

Despite the well-known criticisms of the use of ratios and indices in systematic studies as a method of scaling for size (Atchley *et al.*, 1976), indices (or ratios) have a long and still ongoing tradition of use in ant systematics. Hence the following indices were calculated in order to empirically examine their taxonomic utility:

- CI** Cephalic index: $100(HW/HL)$
- SI1** Scape index 1: $100(SL/HW)$
- SI2** Scape index 2: $100(SL/HL)$
- EI** Eye index (or ocular index): $100(EL/HW)$
- AI** Alitrunk index: $100(MH/WLA)$
- SPI** Spinal index: $100(ISPL/HW)$
- FI1** Femur index 1: $100(FW/FL)$
- FI2** Femur index 2: $100(FL/HW)$

The composition of the data sets used in the analyses are discussed in section 3.6. Statistical analyses were performed using SAS, version 5.12 at the University of Guelph and version 6.03 at Carleton University. All essential documentation for the procedures used (CANDISC, DISCRIM, REG, STEPDISC) can be found in the SAS/STAT User's Guide, Release 6.03 Edition (SAS Institute, 1988). SigmaPlot version 4.0 was used to prepare the bivariate plots in Figures 15–24.

3. RESULTS AND DISCUSSION

3.1. Genetic Delineation of Populations: A Summary

I have classified the colonies in the fulva-rudis-texana complex analyzed in this study into ten distinct forms on the basis of genetic markers. Among the ten forms, nine different basic karyotypes could be recognized easily (see section 3.2). This was exclusive of certain supernumerary chromosome variation or chromosomal abnormalities, and does not include one colony interpreted as having an interspecifically mated queen (these are discussed in sections 3.3 and 3.10). The basic karyotypes differ in chromosome morphology and/or chromosome number. There are two distinct $n = 18$ karyotypes (one belonging to fulva) and two distinct $n = 21$ karyotypes. There are single karyotypes for each of the haploid numbers 16, 17, 19, and 20. In addition, only a single $n = 22$ karyotype could be recognized, although it seemed fairly obvious from field observations and morphological habitus that the $n = 22$ karyotype was common to two species. This was confirmed with electrophoretic data (see section 3.4). The simplest explanation (and the one taken here) is that these forms represent distinct species with varying degrees of morphological differentiation. However, the evidence that each of these forms represents a single distinct biological species is not uniformly strong, and in several cases further analysis will be necessary to further support or refute the hypothesis of species status. The question of how existing names should be assigned to the genetically delineated species is addressed in section 3.12. Since four of the species are undescribed and even the interpretation of each form as a separate species is provisional in some cases, I have assigned code names to each form except for fulva, based on the haploid chromosome number of the basic karyotype. The codes are N16, N17, N18, N19, N20, N21a, N21b, N22a, and N22b. A single large metacentric chromosome in the karyotype of N21a distinguishes it from N21b; in section 3.12 I will argue that N21a is miamiana and N21b is texana. Among the two $n = 22$ chromosome

forms with very similar karyotypes, the code N22a is applied to the most common form (likely the true rudis) and the code N22b applies to the rarer form diagnosed on the basis of a distinct isocitrate dehydrogenase allele. In section 3.12 I argue that the name picea is best applied to N18 (it should be noted here that fulva also has a morphologically distinct $n = 18$ karyotype) and N20 is carolinensis. The forms N16, N17, N19, and N22b likely represent undescribed species.

The use of these codes gives each form (putative species) a unique identifier that is not dependent on its status as a separate species, and will not be affected by possible future changes in nomenclatural interpretations when all relevant types have been studied. However, for those who prefer to use latinized names when available, these have been included liberally in conjunction with the code names. It should be noted that carolinensis and picea are currently recognized as being subspecies names; the formal elevation of these names (as well as other nomenclatural changes and the description of new species) will be done in a separate paper.

In general, the genetically delineated forms corresponded to the impressions gained in the field, particularly where sympatric colour forms were present. Creighton (1950) emphasized the importance of field work in ant systematics, and I would concur with his sentiments. I would add that the impressions obtained from rearing a large number of colonies in the laboratory is also of value in systematic research.

3.2. The Basic Karyotypes: Analysis and Evolution

A representative karyotype is illustrated (Figs. 1, 2) for each of the ten genetically delineated forms in the fulva-rudis-texana complex and the three outgroup species (lamellidens, tennesseensis, treatae). The chromosomes were mounted in decreasing order of length (or mean length of pairs in a diploid karyotype), based on the measurement data provided by CHROMPAC. An idiogram of the haploid karyotype was prepared using this measurement data for each of the representative karyotypes (Figs. 5–7); since the chromosomes in the idiograms are also ordered by decreasing size, there is a one-to-one correspondence between the chromosomes in the photographed karyotypes and the idiograms. Of the additional karyotypes illustrated (Figs. 3, 4), only the hybrid karyotype in Figure 3e was measured and depicted with an idiogram (Fig. 5). Separate five-micron scale bars are presented on the karyotypes in Figs. 1–3, since the photographic prints differ in scale by as much as 12%. The chromosome lengths in all idiograms have been standardized by percentage length of the haploid genome.

Macgregor and Varley (1988) suggested that idiograms should be prepared from the mean values of data obtained from several preparations from several individuals, rather than from a single preparation. Bogart and Tandy (1981) measured three metaphase spreads for each species of frog. In this study, it was only possible to measure a single spread of each species (two spreads from the same N16 individual were measured, but only one of these was used in the preparation of the idiogram). However, for each species the representative karyotype does seem to be characteristic of the numerous spreads examined (see section 3.14 for information on colonies karyotyped), at least for the characters considered diagnostically important.

How reliable are the individual measurements on which the idiograms are based? The absolute lengths of the chromosome arms varies tremendously with the mitotic stage (e.g., see Figs. 1f and 3a for two karyotypes of N20), and to a much lesser degree the relative lengths of

the chromosome arms can also vary with the mitotic stage due largely to the differential rates of condensation for euchromatin and heterochromatin. There will be some variation in arm lengths between members of a homologous pair of chromosomes (even excluding various types of centromere position and arm length polymorphisms) due to differential condensation and the way a chromosome arm folds or twists. Furthermore there is measurement error when using any measuring device including the digitizing system of CHROMPAC. Since only one spread of each species was measured, it is not possible to obtain an estimate of variation among individuals or among stages of chromosomal condensation. However, I believe it is possible to put a minimum bound on the standard error in estimating the relative length of each chromosome by examining the variation between pairs of homologous chromosomes in diploid karyotypes.

To do this, I performed a one-way Analysis of Variance (ANOVA) on the relative length data for the individual chromosomes in each diploid karyotype (N17, N19, N20, N21a, N21b, N22a, tennesseensis), partitioning the total sum of squares among chromosomes into among and within chromosome pair sources. The mean square error (MSE) thus estimates the variance component due to variation between homologous chromosomes, whether due to measuring error or real differences in the lengths. The square root of the MSE gives a standard deviation for the estimate of the true relative chromosome length for a single chromosome in a diploid set, and multiplying this value by the square root of two gives a standard error for a single chromosome in a haploid set obtained by "haploidizing" a diploid set. A 95% confidence bound can be obtained by multiplying these values by 1.96.

The results of the ANOVA's were as follows: N17, MSE = 0.0591, 17 d.f. (degrees of freedom); N19, 0.0141, 19 d.f.; N20, MSE = 0.0719, 20 d.f.; N21a, MSE = 0.0226, 21 d.f.; N21b, MSE = 0.0218, 21 d.f.; N22a, MSE = 0.0691, 22 d.f.; tennesseensis, MSE = 0.03067, 13 d.f. The pooled MSE is 0.041756 based on 133 d.f.; from this, the sample standard deviation

for estimating the relative length of a single chromosome in a diploid set is at least 0.2043% and the sample standard deviation for estimating the relative length of a single chromosome in a haploidized set is at least 0.2890%. For an idiogram prepared from a haploid karyotype, each chromosome measured is, on average, estimating twice the relative length of a single chromosome in a diploid set, so it seems reasonable that the sample standard deviation is at least $2(0.2043) = 0.4086\%$. A minimal 95% bound on error is obtained by doubling these values. Again it must be stressed that these represent minimal values, since all sources of variation are not represented.

The nature of the variation is neither entirely random nor uniform. Some chromosomes are obviously folded or contorted in some way, and the effect of this can be considered. The variance probably increases with the size of the chromosome, but not in direct proportion. For idiograms prepared from haploidized diploid karyotypes, there is a possibility of mismatched pairs, but it is difficult to say if on average this will increase or decrease the estimated variance. For comparisons of karyotypes between different species, differences in DNA content may distort the relative size between homologous chromosomes. The ability of constitutive heterochromatin to grow relatively quickly can also obscure the size comparisons between homologous chromosomes. The analysis of short arms (assumed to be largely heterochromatic) presented in Table 3 partially addresses this problem; the adjustment factor using the tennesseensis karyotype as a standard is a multiplication factor for relative length data that attempts to correct for the growth of the heterochromatic short arms. Imai (1975) noted that the use of length data overestimates the relative contribution of short arms to the genome, and Bogart (pers. comm.) pointed out that there is a tendency to overmeasure the length of very short arms with the CHROMPAC digitizing system. These considerations should be kept in mind in the following discussion.

Although no C-banding protocol was followed in this study, the karyotypes often showed banding patterns (e.g., Figs. 1b,d,e) which were similar to the bands interpreted as C-bands by Imai *et al.* (1977). The most darkly stained regions of chromosomes were usually in the short arms of chromosomes interpreted as acrocentrics and near the centromeres, which is consistent with the usual pattern of the distribution of heterochromatin. These banding patterns were used to help pair homologous chromosomes in diploid karyotypes, and they were also used to help determine if a chromosome classified as "m" or "sm" by CHROMPAC might really be better interpreted as a pseudoacrocentric. However, none of the critical diagnostic characters used to identify a karyotype or make phylogenetic inferences depend on this banding.

As discussed in section 2.2, CHROMPAC classifies a chromosome as "m", "sm", "st", or "t" strictly on the basis of arm length ratio. It was apparent from an overview of the karyotypes and measurement data that many of the chromosomes that appeared to have "single arms" (that is, probably a single euchromatic arm) were classified as st, sm, or even m, particularly if the chromosome was small. Hence I obtained a frequency distribution for the relative arm lengths of the chromosomes in the haploid sets illustrated in Figures 5–7 (the N17 $\times$ N22b hybrid set was excluded). This gave 494 relative arm lengths. There was a peak with a mode at about 1.2%, a strong antinode at about 2.0%, and another peak, flatter and broader than the first, with a mode between 3.1 and 4.0. All arms with a relative length less than 1.6% were short arms. I thus choose to classify chromosomes with a short arm length of less than or equal to 1.50% as acrocentric (A) chromosomes. For chromosomes with a short arm length of more than 1.50%, decisions were made on an individual basis as to whether a chromosome was an acrocentric, pseudoacrocentric (A^M), subtelocentric (ST), submetacentric (SM), or metacentric (M). I believe that this conforms fairly well with the usage of Imai *et al.* (1977, 1988a), although I do not recognize a telocentric (T) category, other than in the strict measurement sense as

provided by CHROMPAC. All CHROMPAC classifications are labelled as such or identified by the appropriate lower-case abbreviation; otherwise, the terms used refer to the classification system modified from Imai *et al.* (1977, 1988a). The following abbreviations are used in the ensuing discussion: R_c = relative length of the complete chromosome, R_L = relative length of the long arm, and R_s = relative length of the short arm.

One of the most distinctive karyotypes is that of N16 (Figs. 1a, 5). Five metacentric chromosomes (#1, 2, 5–7) which are subequal in size constitute approximately 40% of the genome (relative length data for illustrated karyotype: $9.04 + 8.69 + 7.79 + 7.31 + 7.10 = 39.39\%$; for a less condensed karyotype from the same individual: $9.95 + 8.68 + 8.41 + 7.32 + 7.02 = 41.38\%$). I classify the other chromosomes as acrocentrics ($R_s = 0.60–1.32\%$); these are classified as t's (#3, 4, 10) or st's by CHROMPAC. The two large acrocentrics (#3, 4) which are subequal in size with the metacentrics are another distinctive feature of this karyotype; this pair of large acrocentrics is shared only with the N18 and possibly the N21b forms.

The karyotypes of N17 (Figs. 1b, 5) and N18 (*picea*; Figs. 1c, 5) are very similar, except that N17 has a large metacentric (#1) that N18 does not have and N18 has a large acrocentric (#2 or #3; one is assumed to be homologous to #3 of N17) and an extra smaller acrocentric (e.g., #12) that N17 does not have. It seems likely that the large metacentric of N17 can be homologized with the extra acrocentrics of N18 by hypothesizing a Robertsonian rearrangement (fission or fusion). In addition to the large metacentric, N17 has three other "true" metacentric chromosomes (#2, 4, 5) which are likely homologous with chromosomes #2, 4 and one of #5–6 of N18. Chromosome #6 of N17 is classified as m by CHROMPAC but appears to be a pseudoacrocentric, and is likely homologous with one of #5–6 (with #5 favoured) of N18. The rest of the chromosomes can be classified as acrocentrics. In some (but not all) N17 karyotype

preparations a very strong secondary constriction is present on one of the chromosomes (Fig. 5a). This constriction is likely associated with a nucleolar organizer (NOR) site, but this needs to be confirmed using NOR-specific silver staining.

Although the karyotypes of fulva (Figs. 1d, 5) and N18 (picea) share the same haploid number of 18, they differ in some critical features. The most obvious difference is that the two large acrocentrics of N18 (#2, 3) are absent in fulva. The largest metacentric chromosome (#1 in both) is similar in relative length (8.38% in fulva, 8.48% in N18) and centromere ratio (1.24 in fulva, 1.09 in N18). However, although chromosomes #2 and #3 do not differ much in relative length (7.48% and 7.23% in fulva, 8.31% and 8.04% in N18), they do differ greatly in their centromere ratios (1.84 and 2.29 in fulva, 13.56 and 7.77 in N18). Furthermore, although fulva's chromosome #2 might be a pseudoacrocentric (it is classified as sm by CHROMPAC), chromosome #3 appears to be a real submetacentric with two euchromatic arms, as the short arms separate relatively early in metaphase. This highly distinctive chromosome is not obviously homologous with any other chromosome in the fulva-rudis-texana complex, and is usually very easy to spot in a metaphase plate of fulva (e.g., Fig. 4b, arrows). Chromosomes #4, 6, 8, 9 are classified as m's by CHROMPAC; of these, #8 and #9 may be pseudoacrocentrics. Chromosome #12 is a real submetacentric. Chromosomes #5 and #13 also have short arms with $R_f > 1.50$; these are both probably pseudoacrocentrics. The other chromosomes (#7, 10, 11, 14 ~ 18) are classified as acrocentrics.

The karyotypes of N19 (Figs. 1e, 6) and N20 (carolinensis; Figs. 1f, 6) are easily distinguished from other fulva-rudis-texana complex karyotypes by their possession of a pair of large metacentrics which stand out in sharp contrast to the other much smaller, mainly acrocentric, chromosomes. The only detectable differences between the two karyotypes consist of the possession by N20 of an extra acrocentric chromosome (#20) which is noticeably smaller

than the rest and a size difference for the largest metacentric. The largest metacentric of N19 measured $R_C = 15.79\%$, whereas the largest metacentric of N20 measured $R_C = 12.79\%$. However, in the diploid karyotype of N20 there is an abnormally large difference in the two homologous chromosomes, with the left chromosome of the first pair in Figure 1f measuring 5.67% in relative length (of the diploid karyotype) and the right chromosome measuring 7.12%. The left chromosome appears to be more condensed and possibly folded in the longer arms, and thus is likely undermeasured; if the relative length of the larger (right) chromosome is doubled, a value of $R_C = 14.24\%$ is obtained. This is still substantially short of the R_C of N19's largest metacentric by 1.55%. Chromosome #20 of N20 measured $R_C = 2.71\%$ and $R_L = 1.87\%$. Chromosome #2 has very close measurements for the two karyotypes ($R_C = 9.96\%$ for N19, 9.95% for N20), and there are no substantial differences in the measurements for chromosomes 3–19 for the two sets. Clearly chromosome #20 of N20 must be derived from chromosome #1 of N19, or else it must have fused with chromosome #1 of N20 to produce the longer chromosome of N19. It is difficult to say how this fission (or fusion) might have taken place, but if it was a fission, I believe it may have come from the long arm in the N19 metacentric, which would then become the short arm of the N20 metacentric. Note that on the long arm of the left largest metacentric of N19 (Fig. 1e) there appears to be some form of light constriction. More research is required to establish the mechanism of this chromosomal change.

I consider the presence of the smallest acrocentric chromosome of N20 highly significant, as it is also found in N21a (miamiana), N22a (rudis), and N22b, but absent in all other members of the fulva-rudis-texana complex and also absent in the three outgroup species karyotyped. It was always found in karyotypes of these forms, and thus does not appear to be a dispensable element. Thus I consider this small acrocentric to be a clear synapomorphy, uniting the four forms in which it is found as a monophyletic group.

Chromosomes #3 of N19, and #3 and #6 of N20, have short arms for which $R_s > 1.50\%$ (1.75%, 1.62%, 1.70% respectively) but can be classified as acrocentrics or pseudoacrocentrics. Chromosomes #5 of N19 and #7 of N20 have substantially longer short arms (2.08%, 2.16% respectively); I prefer to classify these as small metacentrics although they might be pseudoacrocentrics. All other chromosomes in the two forms are clearly acrocentrics.

The single large metacentric ($R_c = 13.10\%$) in the karyotype of N21a (miamiana; Figs. 2a, 6) appears to be homologous to the largest metacentric in the karyotype of N20 (carolinensis). It would seem to be simple to link the second largest metacentric (chromosome #2) of N20 with two acrocentrics of the appropriate size of N21a. However, chromosome #2 of N20 had $R_L = 6.12\%$, which exceeds the R_L of any of the acrocentric chromosomes of N21a by a substantial amount (chromosomes #2, 4, 8, 10 have $R_L = 3.88-3.92$). This seems to be a rather large discrepancy that is difficult to attribute to probable sampling error. One possibility is that part of the long arm of chromosome #2 of N20 has been lost or translocated to another chromosome in N21a. The early metaphase diploid karyotype of N20 in Figure 3a shows strong constrictions at the distal ends of the long arms of the second pair of metacentrics, which might indicate a susceptible breaking point or possible point of fusion. Although further research is necessary to clarify whether there have been extra chromosomal changes between the two karyotypes, it nonetheless is reasonable to conclude that a single fission or fusion event has been the main chromosomal change required to derive one karyotype from the other.

In the rest of the N21a karyotype, the very small acrocentric (#21, $R_s = 2.83\%$, $R_L = 1.73\%$) and small metacentric (#5) appear to be homologues of chromosomes #20 and #7 of N20, respectively. I consider chromosome #2 of N21a to be an acrocentric or pseudoacrocentric ($R_s = 1.87\%$), probably homologous with chromosome #3 of N20. The short arm of chromosome #3 is interesting, having $R_s = 1.53\%$; this chromosome may have undergone a pericentric

inversion, but it makes little difference to the overall impression of the karyotype to classify it as an acrocentric. The rest of the chromosomes (#4, 6–20) can also be classified as acrocentrics, although all of them except #9 are classified as sm or st by CHROMPAC.

The karyotype of N21b (*texana*; Figs. 2c, 7) consists mainly of small to medium sized acrocentrics, and lacks the distinctive large metacentric of N21a (*miamiana*). It also appears to lack the very small acrocentric of N20 (*carolinensis*) and N21a (although in view of possible sampling error this needs to be confirmed); the smallest acrocentric has $R_C = 3.13\%$ and $R_L = 2.39\%$. The small metacentric appears to be present as chromosome #6; this is the only chromosome in this karyotype that I do not classify as an acrocentric or pseudoacrocentric. The two largest acrocentrics (#1, 2) can be derived by a fission of the largest metacentric (#1) of N19 (or alternatively N19's metacentric can be derived by a fusion of the two acrocentrics); indeed, there are very similar banding patterns between the long arm of N19's chromosome #1 and N21b's chromosome #1, and between the short arm of N19's chromosome 1 and N21b's chromosome #2 (Figs. 1e, 2b). There would also need to be a fission or fusion event to link chromosome #2 of N19 with two acrocentrics of N21b. There is some discrepancy in size here, as chromosome #2 of N19 has $R_L = 5.74\%$ and the longest arms of the chromosomes of N21b other than #1 and #2 are those of chromosomes #3 and #4, with $R_L = 4.46\text{--}4.47\%$. Chromosome #13 appears to have been anomalously measured, with $R_C = 4.31\%$ and $R_S = 2.08$; I classify this chromosome as an acrocentric or pseudoacrocentric.

The karyotypes of N22a (*rudis*; Figs. 2c, 7) and N22b (Figs. 2d, 7) are, like N21b, composed almost entirely of acrocentrics. Unlike N21b (*texana*), they both have a very small acrocentric. Chromosome #22 of N22a had measurements of $R_C = 2.50\%$ and $R_L = 1.64\%$, while the next smallest chromosome had $R_C = 3.37\%$. N22b had two unusually small chromosomes as indicated by the measurements, chromosomes #21 ($R_C = 2.55\%$, $R_L = 2.03\%$)

and #22 ($R_C = 2.03\%$, $R_L = 1.61\%$); I believe the small size of chromosome #21 may be a sampling artifact, but this requires confirmation. The most likely candidate to be the homologue of the small metacentric seen in several other forms is chromosome #3 in N22a, but in N22b there is some doubt. Chromosome #11 is the only one classified as m by CHROMPAC, but perhaps a more probable candidate is #7, which has a folded short arm that may have been undermeasured ($R_L = 3.12$, $R_S = 1.39$).

The differences between the N22a (*rudis*) and N22b karyotypes as depicted on the idiograms (Fig. 7), particularly in the relative lengths of chromosomes #1 and #2, may be a sampling artifact. There is a strong possibility that some chromosomes may have been mismatched in the N22a karyotype (hence the relatively large mean square error), including those in the first two pairs. The two largest chromosomes in the diploid set were the left chromosomes in each of the first two pairs; if these were matched, the largest chromosome in the haploid set would have measurements of $R_C = 7.86\%$ and $R_L = 6.32\%$. In comparison, the largest chromosome of N22b had measurements of $R_C = 8.48\%$ and $R_L = 7.69\%$. It was my impression from examining numerous karyotypes of these two forms that the largest acrocentric of N22b was usually more distinctive than the largest acrocentric of N22a, but this needs to be established statistically by measuring several spreads of each form, as Angus (1982,1983) did in his studies of sibling species in the European hydrophilid genus *Helophorus*. In any event, this would be difficult to use as a diagnostic character to separate N22a and N22b. The additional karyotypes of N22a (Figs. 3b,c) and N22b (Fig. 3d) do suggest, however, that the idiograms are reasonably accurate.

The sum of the relative lengths for the long arms of the two largest chromosomes of N22b is $7.69 + 5.30 = 12.99\%$. This value is compatible with linking these two chromosomes with the largest metacentric (chromosome #1) of N20 and N21b through a Robertsonian event,

although there are some differences in the match of individual arm lengths. For N22a, the sum of the R_L values for the two largest chromosomes is $5.62 + 5.29 = 10.91\%$, which is much less compatible with hypothesizing the same chromosomal change. Nonetheless it seems reasonable that such a change must have occurred, although there may have been subsequent changes which affected the lengths of the arms to some degree.

The large metacentric in the karyotype of lamellidens (Figs. 2e, 6) is similar in size ($R_C = 13.31\%$, $R_L = 6.94\%$) to the largest metacentric of N20 (carolinensis) and N21a (miamiana). The karyotype is superficially similar to that of N21a, although it only has $n = 19$, but the chromosomes of the two forms do not homologize well. Chromosomes #2 and #3 are small metacentrics, of which at least one of them is usually quite noticeable in a metaphase plate. Chromosomes #6, 9, 11, 14, 15, and 17 were all classified as m's by CHROMPAC (all $R_s \geq 1.50$) and several other chromosomes classified as sm's had $R_s > 1.50$ (#4, $R_s = 2.22$; #5, $R_s = 1.87$; #7, $R_s = 1.79$). The smaller chromosomes ($R_L < 7\%$) of members of the fulva-rudis-texana complex appear to have longer long arms and shorter short arms than those of lamellidens. Since lamellidens is a highly distinctive species, with no obvious close relative, the longer short arms may reflect a long evolutionary isolation, giving more time for the centering of the centromeres through pericentric inversion and the tandem growth of heterochromatin, as predicted by the minimum interaction hypothesis (see section 1.4).

The $n = 13$ karyotype of tennesseensis (Figs. 2f, 7) is highly distinctive, with the eight largest chromosomes being metacentrics of $R_C > 7.0\%$ and $R_s > 3.0\%$. Chromosome #10 is also classified as an m by CHROMPAC, but I have classified this chromosome as a pseudoacrocentric ($R_s = 1.84$), partially on the basis of a darkly stained short arm in an unillustrated spread. Alternatively, this chromosome may be homologous to the small metacentric

found in many fulva-rudis-texana complex karyotypes. The rest of the chromosomes are acrocentrics.

The tennesseensis karyotype shows several features that invite speculation concerning karyotype evolution in the fulva-rudis-texana complex. Chromosome #1 ($R_C = 16.01\%$) is very close in size to chromosome #1 of N19, although the centromere is further off center. Chromosome #2 ($R_C = 11.49\%$, $R_L = 6.13\%$) compares very well in size and rather favourably in centromere position with chromosome #1 of N17 ($R_C = 11.41\%$, $R_L = 6.90\%$). The correspondence is excellent between the five metacentrics of N16 and chromosomes #3, 4, and 6–8 of tennesseensis, and between chromosome #8 of N16 and chromosome #9 of tennesseensis. Indeed, with a pericentric inversion in chromosome #1 of tennesseensis to better approximate the centromere position of chromosome #1 of N19, it is reasonably easy to derive all of the karyotypes of members of the fulva-rudis-texana complex, except possibly fulva, from the tennesseensis karyotype or something close to it.

The most distinctive element of the treatae karyotype (Figs. 2g, 7) is chromosome #2, a medium-sized metacentric. Otherwise the karyotype appears to be similar, at least superficially, to that of N21b, with only a pericentric inversion of chromosome #2 in N21b required to produce chromosome #2 of treatae. Chromosomes #5 and #8 can be classified as small metacentrics, although the compositions of the small arms are unknown, while the rest of the chromosomes can be considered acrocentrics.

With the exception of one anomaly, the karyotypic results of Crozier (1975, 1977) are highly concordant with my own. The light brown form with $n = 16-18$ and the dark brown form with $n = 18$ that Crozier (1975) found around Ithaca, New York are clearly N16 (undescribed) and N18 (picea), respectively (with the former showing supernumerary chromosome variation). I also interpret the $n = 18$ "Montane" population from Black Rock

Mountain State Park, Georgia belonging to N18 (picea). Under this interpretation, picea is found throughout the Appalachians, particularly at higher altitudes (see section 3.14), and much of northeastern North America. Crozier (1977) believed that the karyotypes from New York and Georgia differed by "a number of pericentric arrangements". Although I can detect some small differences between some populations of N18, I cannot see anything that would lead me to believe they are not conspecific. However, it must be admitted that the quality of the karyotypes that I obtained from the Georgia population did not allow a high degree of resolution.

The $n = 20$ species of Crozier (1975, 1977) is clearly N20 (carolinensis), and the $n = 22$ "Montane" species is N22a (rudis). Crozier (1977) also gave the karyotype of fulva; as well, the colony from Alexander Springs, Florida identified as "rudis-group (?miamiana)" with a karyotype similar to fulva is, in fact, fulva itself (see section 3.7 for a morphometric identification of a voucher specimen). The karyotypes of lamellidens and treatae illustrated by Crozier (1977) are very similar to those I have illustrated here.

The anomalous result is the $n = 22$ karyotype with one very large metacentric for the "dark 'rudis' from Alachua County, Florida" illustrated by Crozier (1977; his Fig. 18A). This karyotype appears to be identical to that of N21a (miamiana), except that it has one extra pair of small acrocentrics in the diploid karyotype. Although Crozier used four colonies of this form in his electrophoretic analysis, it is not clear how many colonies or individuals were karyotyped. I believe his illustrated karyotype is simply that of N21a with supernumeraries (see section 3.3). Future examination of vouchers should provide strong evidence to either support or refute this hypothesis.

Although Crozier (1977) considered the karyotypes of fulva and N18 (picea) karyotypes to be very similar, there are some substantial differences between them. The superficial similarities between the karyotypes may have been exacerbated by Crozier's use of a long

colchicine pretreatment, which greatly increases the number of metaphases available for counting but tends to condense the chromosomes substantially. Since I have not used colchicine at all, it has been possible to obtain higher resolution of the chromosome morphology in many cases.

It seems clear that Robertsonian changes (either centric fissions or fusions) have constituted the major mechanism of karyotype evolution in the fulva-rudis-texana complex, and indeed throughout the genus Aphaenogaster in general. The new karyotypes presented in this thesis (tennesseensis, N17, N19, N21b, N22b) supply additional data in corroboration of the conclusions of Imai *et al.* (1977, 1984b). Without good C-banded karyotypes, it is difficult to determine whether some chromosomes have one or two euchromatic arms. Nonetheless it appears that the number of euchromatic arms, or fundamental number (FN), in Aphaenogaster karyotypes is in the low twenties, although there is considerable variation in chromosome number (see section 1.4). Aphaenogaster longiceps and A. beccarii must have $FN \geq 23$ (likely 23 or 24), while the lowest FN value may be $FN = 21$ (e.g., tennesseensis). Within the fulva-rudis-texana complex, N16 clearly appears to have $FN = 21$, and this is likely the FN value for N17, N18 (picea), N19, and N21b (texana). The very small acrocentric present in the karyotypes of N20 (carolinensis), N21a (miamiana), N22a (rudis), and N22b is associated with an increase in the FN value by one. As previously discussed, the presence of this very small acrocentric constitutes the strongest chromosomal synapomorphy as judged by outgroup comparison. The FN value for fulva is very difficult to determine.

Minimal transitional steps between the karyotypes with the very small acrocentric present as well as those of N19 and N21b (texana) are summarized in Figure 8a. No polarity is assigned to the direction of fission or fusion events. If centric fissions are much more likely than centric fusions as predicted by the minimum interaction hypothesis (see section 1.4), the "root" karyotype is that of N19. Among the other karyotypes, those of N17 and N18 (picea) can be linked by a

single Robertsonian change. Only N16 and N18 share a pair of large acrocentrics (chromosomes #3, 4 of N16 and #2, 3 of N18), but there are numerous other small differences between these karyotypes. One of these acrocentrics (at least for N18) is likely homologous with the long arm of chromosome #1 of N17. The extreme morphological similarity of N17 and N18, contrasted with several small but significant differences in both morphology and karyotype between this pair and N16, gives at least some support to the idea that N17 and N18 are sister forms. If the presence of two large acrocentrics is interpreted as being a derived character state, it can be hypothesized that one of them subsequently underwent a centric fusion with a smaller acrocentric to create chromosome #1 of N17. Although the relationship between N17 and N18 seems clear, their relationship with N16 should be considered tenuous. It is even more difficult to connect the N16 + N17 + N18 group to the N19 + N20 + N21a + N21b + N22a + N22b group. A highly speculative connection can be made by hypothesizing an ancestral karyotype similar to that of tennesseensis.

The relationship of Aphaenogaster tennesseensis to the members of the fulva-rudis-texana complex is intriguing. It has been conjectured that tennesseensis is a temporary social parasite on (at least some) members of the fulva-rudis-texana complex (Creighton, 1950; Carroll, 1975). Evidence in support of this hypothesis includes the small size and unusual morphology of the queen, and the finding of some mixed colonies of tennesseensis and a fulva-rudis-texana complex member. Although the identities of the hosts need to be reappraised in light of the results of this thesis, the wide distribution of tennesseensis (southern Ontario to northern Florida (personal collecting), west to northern Texas (Moody and Francke, 1982), Oklahoma and Wisconsin (Creighton, 1950)) indicates that it must utilize several species of hosts. A loose form of Emery's rule states that social parasites are fairly closely related to their hosts (Hölldobler and Wilson, 1990). At least on the basis of undifferentially stained chromosomes it is fairly easy to

derive the basic karyotypes of the fulva-rudis-texana complex members (except fulva) from a hypothesized "ancestral" karyotype similar to that of tennesseensis. It could be hypothesized that the separation of tennesseensis and a fulva-rudis-texana complex ancestor preceded speciation events in the complex. [It might be conjectured that temporary social parasitism by tennesseensis has, in fact, been a factor in the evolution and maintenance of species diversity within the fulva-rudis-texana complex. This suggests an examination of the concordance of the presence of social parasites with sibling species complexes in ants.] A karyotype similar to that of N16 (and then leading to N18 and N17) can be obtained by centric fissions of chromosomes #1, 2, and 5 of tennesseensis. A karyotype similar to that of N19 can be derived from that of tennesseensis by a pericentric inversion of chromosome #1 of tennesseensis and centric fissions of its chromosomes numbered 3–8. It would be most parsimonious if the centric fission of chromosome #5 of tennesseensis occurred on only one occasion. This scenario of karyotype evolution is summarized in Figure 8b.

The phylogeny presented should be interpreted only as a working hypothesis subject to further testing. Phylogenetic inference using unbanded karyotypes is a somewhat risky endeavor, as a substantial portion of chromosomal rearrangements may not be visible (e.g., paracentric inversions, reciprocal translocations of approximately equal length arms). Baker *et al.* (1987, p. 69), reviewing the role of chromosomal banding in elucidating mammalian phylogenies, stated that "nondifferentially stained karyotypes provide little estimate of genetic homology of chromosomes." They cited studies where the number of chromosomal rearrangements required to explain the observed chromosomal variation was much greater when the chromosomes were banded; for example, in the phyllostomid bats, over five times as many rearrangements were required to explain the G-banded karyotype differences as were necessary for the unbanded karyotypes. However, in some other mammalian taxa (e.g., carnivores, primates, phyllostomid

bats, vespertilionid bats), the scenarios of chromosomal evolution indicated by nondifferentially stained karyotypes and G-banded karyotypes were essentially the same (Baker *et al.*, 1987).

Homoplasy due to convergence, parallelism or evolutionary reversal is another potential pitfall in the estimation of the phylogeny (Futuyma, 1986). Chromosomal breakage points and types of chromosomal rearrangements can be expected to be highly nonrandom in their occurrence. If there is a drive towards centric fissions, as predicted by the minimum interaction hypothesis, then the "uncoupling" of euchromatic arms should be expected to occur along independent lines. The similarity of the *treatae* karyotype and those of N21b (*texana*), N22a (*rudis*), and N22b is likely due to convergence, whereas the similarity of the N21b karyotype and those of N22a and N22b is likely due to parallelism. Indeed I am not convinced that N22a and N22b are sister species, despite the similarity of their karyotypes and many morphological features (see sections 3.6 and 3.8 and Fig. 20b). Centric fusions, reversals in the direction towards fissions, might be more likely to occur due to telomeric instability when the short arms of newly created acrocentrics are not yet "capped" by the tandem growth of heterochromatin (see section 1.4).

Differential staining of the chromosomes (along with karyotyping further outgroup species) will almost certainly improve our understanding of karyotype evolution in the *fulva-rudis-texana* complex. As previously noted (section 1.4), the techniques of C-banding and silver staining for nucleolar organizing regions have already been performed on ant chromosomes. Time constraints prevented the application of these procedures during my thesis research, but this is an obvious avenue for future investigation. The most useful banding procedure for establishing chromosomal homologies in some groups, such as mammals, is G-banding (I include the very similar Q-banding here). Some investigators have reported obtaining clear G-banding patterns in insects (e.g., Lester *et al.*, 1979, on the house fly *Musca domestica*; Steiniger and Mukherjee,

1975, on the mosquito Aedes albopictus). However, the so-called G-bands found in insects may not be equivalent to those found in mammals. Holmquist (1989) has hypothesized that G-bands are a derived chromatin character state that evolved within the early chordate lineage, and noted that Drosophila does not have G-bands or Q-bands. If so, true G-banding of ant chromosomes may not be possible, but other procedures such as in situ hybridization using nucleic acid probes (Sessions, 1990) could be highly informative. The development of cell culture techniques for ant tissues should allow more rapid development of differential staining procedures for ant chromosomes.

3.3. Supernumerary and Other Chromosome Variation

Supernumerary chromosome variation was observed in individuals of at least nine (likely eleven) colonies in this study. Although the data is not extensive, it does suggest that two types of supernumeraries are present in at least some of the forms in the fulva-rudis-texana complex. These are (1) supernumeraries that occur in the somatic cells (brain tissue), and (2) supernumeraries that appear only in the testes. The appearance of these supernumeraries causes few problems in identifying the form to which a colony belongs on the basis of its karyotype, as they are either small acrocentrics similar to others in the basic karyotype, or smaller chromosomes or fragments.

The following gives a complete list of the data for which it is likely that supernumerary chromosomes were present. Data are listed by form, locality (see Table 2 for codes), and colony number. Raw data on counts and material karyotyped is presented by slide number in brackets.

N18 (picea). QN3: A223 (#448, 12 counts $n = 19$, 2 counts of $n = 18?$, testes; 3 slides had $n = 18$, all male brains).

N20 (carolinensis). GA1: A312 (#607, 5 counts $2n = 43$, worker brain). SC2: A295 (#590, 1 count $2n = 41$, good spread, worker brain).

N22a (rudis). KY1: A073 (#324, 7 counts $n = 23$, 2 counts $n = 22$, 1 count $n = 20$, testes; #392, 18 counts $n = 28$, 2 counts $n = 27-28$, 1 count $n = 27$, testes; #464, 13 counts $n = 24$, 1 count $n = 24?$, 1 count $n = 23$, testes; # 526, 11 counts $n = 22$, 1 count $2n = 44$, 1 count $n = 22$ + possible small fragment?, 1 count of $n = 17$ but chromosomes likely lost, male brain).

NC2: A279 (#668, 5 counts $2n = 46$, worker brain). NJ2: A160 (#346, 7 out of 7 counts $n = 22$, male brain; #433, 12 out of 13 counts $n = 26$, 1 count $n = 26?$, testes; #428, 6 counts $n = 26$, 1 count $n = 27?$, testes; #459, 10 counts $n = 26$, 4 counts $n = 25$, 1 count $n = 24$, testes, tissue left in hypotonic solution too long, chromosomes may have been lost in $n < 26$ spreads;

#477, 4 counts $n = 22$, 1 count $2n = 44$, male brain; #476, 5 counts $n = 22$, 1 count $n = 22?$; #534, 5 counts $n = 22$, male brain). NJ4: A157 (#278, 6 of 11 counts $2n = 44$, no good evidence of supernumeraries although 1 count of $2n = 44-45$, queen brain; #352, 4 counts $n = 22$, 1 count $2n = 44$, male brain; #350, 14 counts $n = 22$, 6 counts $n = 23$, 1 count each of $n = 22?$ and $n = 23?$, 1 count of two inseparable spreads of $2n = 45$, testes; #351, 7 counts $n = 22$, 1 count $n = 23$, 1 count $n = 23?$, testes). TN2: A334 (#568, 3 counts $2n = 46$, 1 count $2n = 46?$), A336 (#639, 2 counts $n = 22$, 1 count $2n = 44$, 1 count $n = 23$, only slight doubt on last count, worker brain); A337 (#604, 5 counts $2n = 45$, 1 count $2n = 43-44 + 1$ fragment, extra chromosome is clearly a fragment, worker brain).

Summarizing this information, it can be noted that in four of the colonies (N18: A223; N22a: A073, A157, A160), the supernumeraries are restricted to the slides of testes; the slides of male brains (or one queen brain for A157) never had supernumeraries. Supernumerary variation in the brain was noted in the other six colonies (N20's A295, A312; N22a's A279, A334, A336, A337).

Of the colonies with supernumeraries restricted to the testes, A157 shows evidence of only an occasional single supernumerary. However, the evidence in the other three colonies is quite clear, particularly for A073 and A160. Of the four slides of A073, the three slides of testes had varying numbers of supernumeraries (1-7), with counts of $n = 23$ (Fig. 4d), $n = 24$ (Fig. 4e), and $n = 28$ (Fig. 4f). In contrast, of the six slides of A160, the three slides of testes all had four supernumeraries (count of $n = 26$). The single slide of testes made for A223 had a single supernumerary ($n = 19$). In most cases the number of supernumeraries were reasonably constant within a slide, with the exception of colony A157. It was not possible to discern which of the chromosomes were the supernumeraries; they are small acrocentrics similar in size to others in the basic karyotype.

The restriction of the supernumeraries to the testes does not appear to be a chance event. For A160 alone, the probability that all of the brain slides would not have supernumeraries and all of the testes slides would, if it were a random allocation, is $p = 0.05$. If the data from A073 were combined, $p = 0.0048$, and if the data from N18's A223 were pooled, $p = 0.00029$. This suggests that a mechanism for selectively accumulating supernumeraries in the germ tissue and shedding them from the somatic tissue is operating in these colonies. It should be noted that probably all males karyotyped in colonies A073, A157, A160, and A223 were produced from worker-laid eggs. Although there is no good reason to believe that males produced from queen-laid eggs would not exhibit similar supernumerary phenomena, this possibility merits future investigation.

The supernumeraries found in the brains often appeared to be dots or fragments. Spreads of A334 (Fig. 4g) show a small dot which is clearly a supernumerary (small arrow); another small chromosome or fragment is also present (this may be indicated by the large arrow, but this is uncertain). This colony was one of four N22a (rudis) colonies collected at TN2 in a small patch of forest; only colony A335 (5 counts of $2n = 44$) showed no evidence of supernumeraries. The evidence of a supernumerary in one spread of A336 is poor, but an extra chromosomal fragment is clearly present in all six spreads observed for A337.

The three extra chromosomal elements in the spreads of A312 are also clearly fragments. This was one of five colonies of N20 (carolinensis) karyotyped (1 slide each) from GA2, the site where Crozier (1977) had previously found supernumerary chromosome variation. This site would appear to be an excellent locality to study the variation of the supernumeraries within and among colonies, and examine their transmission in the germ line.

It is uncertain whether the supernumerary chromosomes observed in this study are B chromosomes, in the sense defined by Jones and Rees (1982). They differentiate B chromosomes

from other types of chromosomes by two criteria: their distribution within species and their pairing behaviour at meiosis. B chromosomes occur in some individuals in some populations, but not in most individuals; they are dispensable, and may even have deleterious effects. At meiosis, B chromosomes will pair with one another and form chiasmata, but they will never pair with members of the normal (A) chromosome set. The cases of supernumerary chromosome variation observed in the fulva-rudis-texana complex clearly satisfy the first criterion, but no information is available on their pairing behaviour at meiosis.

Jones and Rees (1982) gave six further criteria that are often characteristic of B chromosomes, although not invariably. To paraphrase their list:

- (1) B chromosomes differ morphologically from A chromosomes; they are often highly heterochromatic (most animal species) and in most species they are smaller than the A chromosomes.
- (2) B chromosomes display non-Mendelian inheritance.
- (3) Only rarely do B chromosomes carry nucleolar organizers.
- (4) The frequencies of B chromosomes within an individual can vary from organ to organ due to nondisjunction at anaphase of mitosis.
- (5) In high numbers they depress fertility and reduce growth.
- (6) They carry no genes with major effects on the phenotype.

They further note that B chromosomes establish themselves in populations with some persistence. This differentiates B's from small chromosome fragments that arise and show little persistence in the population although they may be transmitted to offspring for a few generations. It is likely that the supernumeraries observed in this study that were found restricted to the testes are true B chromosomes, whereas at least some of the supernumeraries found in the brains may not be.

It is known that P chromosomes can be eliminated in certain tissues and accumulated in others; there is a tendency towards accumulation, particularly in the germ line (Jones and Rees, 1982). The work of Nur (1963) is a classic analysis of such a system in the grasshopper Calliptamus palaestinus. Imai (1974) studied supernumerary chromosomes, which he called B chromosomes, in the myrmicine ant Leptothorax spinosior. He found that the supernumeraries were restricted almost entirely to the male germ cells (up to 12 supernumeraries added to the basic $n = 12$); they were rarely found in the somatic cells of any caste or in female germ cells. His analysis of heterochromatic bodies in female cells suggested that the supernumeraries were maintained in the oocytes but almost entirely eliminated from nurse cells.

Supernumerary chromosome variation in the N16 form reported by Crozier (1975) was not found in my study, but this is not surprising given the patchy distribution of colonies with supernumeraries; no attempt was made to collect around Ithaca, NY, where Crozier made his discoveries. In section 3.2 I have suggested that his report (Crozier, 1977) of an $n = 22$ karyotype from Alachua Co., Florida may be due to possible supernumerary chromosome variation in the N21a form. Clearly one must be very careful in using the chromosome number alone to identify a member of the fulva-rudis-texana complex. However, most karyotypes in the complex can easily be distinguished from one another by the morphology of the larger chromosomes. Theoretically it would be difficult to distinguish a karyotype of N22a (rudis) or N22b from a karyotype of N21b (texana) with a small supernumerary, but given the very different range and morphological features of N21b, this is highly unlikely.

Very little other chromosomal variation was detected in this study. A triploid individual (prepupal brain karyotyped, caste unknown, 8 of 8 counts $3n = 48$) was found in a colony of N16 (A202 from ON1). Individual polyploid cells were often noted, with a clear count of $8n = 160$ obtained for one N20 (carolinensis) spread; polyploid cells had previously been recorded in

Aphaenogaster osimensis by Imai and Yosida (1965b). An unusual feature of paired bivalents was noted in some spreads of very young queen brains with other ganglia attached (Fig. 4h), suggesting endopolyploidy (White, 1973). The single individual karyotyped from colony A280 (N22a from NC2) appeared to have a very long heterochromatic short arm on one chromosome. In the single individual karyotyped of colony A347 (N17 from KY2) there were three counts each of $2n = 33$ and $2n = 34$; the missing chromosome appeared to be a real phenomena in two of the spreads. There were a few cases where a minor translocation may have been noted in single spreads, but this was difficult to discern. Overall, other than the supernumerary chromosome variation noted, the karyotypes of each form appeared remarkably consistent at the level of resolution employed, thus making them excellent taxonomic characters.

3.4. Electrophoretic Investigations: A Synopsis

The most important results obtained to date in the electrophoretic investigations are summarized here. In particular, they give solid genetic evidence for the separation of N22a (rudis) and N22b, describe an excellent allozyme for recognizing fulva, and present another enzyme that might indicate widespread gene flow among members of the fulva-rudis-texana complex. (The electrophoretic evidence for the hybrid nature of colony A221 is discussed in section 3.10.) However, an insufficient number of enzymes could be satisfactorily resolved to provide a definitive study of isozyme variation in the fulva-rudis-texana complex. It is suggested that alternative methodologies are likely to be more informative for studying enzyme variation in this complex.

Bernie May considered 29 enzymes to be worthy of future investigation after the initial screening at CLEEG (Table 4). Of these, 26 were run at Carleton University. Three were omitted because of the cost of their staining chemicals and limited potential as judged by the screening at CLEEG (HA and α -MAN showed weak activity, and GR was monomorphic). Eight of them showed at least good systematic potential (AAT, EST-AS-D, GK, GPI, IDH, MDH-1,2, ME, PGM-1,2), but not all of these could be scored consistently (EST-AS-D, GK in part, MDH-1,2). The most useful enzymes are described below.

Isocitrate dehydrogenase (IDH): This enzyme provided definitive evidence that N22b was a separate species from N22a (rudis), and also provided further evidence of the hybrid nature of colony A221. Although IDH is a dimer (May et al., 1988), almost all individuals run were homozygous and thus produced only a single dark band which ran anodally on a "4" gel. On most runs a single light band trailed the dark bands by a fixed distance; this was treated as an artifact. Only the two most commonly found alleles were scored: *Idh*¹ for the common "slow"

allele, and *Idh*² for a "fast" allele (see Fig. 9a). All N22b individuals were homozygous for *Idh*². In contrast, ants from 149 out of 154 other fulva-rudis-texana complex colonies were homozygous for *Idh*¹, and none had an *Idh*² allele. The other five colonies had rare variant banding patterns, but in each case there appeared to be one *Idh*¹ allele present (Fig. 9a, positions 4–7). For the outgroup material examined, lamellidens and treatae clearly scored as *Idh*^{1/1}. For tennesseensis, the very heavy band obtained tended to obscure its position somewhat; it could not be determined if it had the same electrophoretic mobility as *Idh*^{1/1} or ran slightly faster (it was clearly not *Idh*^{2/2}). Since at least two of the three outgroup species were scored as *Idh*^{1/1}, I interpret *Idh*¹ as representing the plesiotypic condition in the fulva-rudis-texana complex and *Idh*² as a uniquely derived character state (autapomorphy) for N22b. The data are not sufficient to say that *Idh*² is fixed in N22b, as 59 separate genomes (30 independent workers) are required to be 95% certain of detecting an alternate allele with a frequency of 0.05 in the population. The probability of not detecting an alternate allele with a frequency of 0.05 in the population with seven independent workers is 0.48; however, colonies from Ontario (5), Indiana (1), and Maryland (1) were included in the analysis.

[Material examined for IDH (at least one individual reliably scored per colony, number of colonies in brackets): N16 (10), N17 (21), N19 (5), N20 (15), N21a (7), N22a (52), N22b (7), A221 hybrid colony, lamellidens (3), tennesseensis (4), treatae (3)]

Aspartate aminotransferase (AAT): This enzyme ran cathodally on a "C" gel, and appeared to be a dimer. Two common alleles were scored: *Aat*¹ produced a faster running homozygous enzyme than *Aat*² (Fig. 9b, positions 1,2). The heterozygote *Aat*^{1/2} also produced an intermediate running band, as expected for a dimer (Fig. 9b, position 3). Except for N22a (rudis), almost all individuals scored were homozygotes. With the exception of some rare variants (discussed later),

all individuals of N16, N17, N18 (picea), N22b, fulva, and the outgroup species lamellidens, tennesseensis, and treatae were homozygous for Aar^1 , whereas N19 and N20 were homozygous for Aar^2 . All individuals of N21a (miamiana) and N21b (texana) examined were homozygotes, but with an unusual geographic pattern. For N21a, workers from nine colonies were scored $Aar^{2/2}$, while a single colony (5 workers scored) had $Aar^{1/1}$. The nine $Aar^{2/2}$ colonies were all "dark" colonies from the northern part of the range of N21a, whereas the single $Aar^{1/1}$ colony (A096) was a "light" colony from southern Florida. For N21b, 10 colonies (at least one worker per colony) from Arizona were all homozygous for Aar^1 , whereas a worker from the Texas colony (A422) was homozygous for Aar^2 .

In N22a (rudis), heterozygotes were fairly common. For the 51 colonies that could be reliably scored for at least one worker (usually only one worker was scored), 2 were scored $Aar^{1/1}$, 14 were scored $Aar^{1/2}$, and 35 were scored $Aar^{2/2}$. Treating these 51 colonies (essentially 51 workers from separate colonies) as representing a random sample of 102 genomes from a single population, estimates for the proportions of the alleles Aar^1 and Aar^2 in the population of 0.18 and 0.82, respectively, are obtained (standard error for each estimate is 0.038). A value for the chi-square goodness-of-fit test statistic of 0.16 (2 d.f., $p > 0.50$) shows little evidence of departure from Hardy-Weinberg equilibrium. Although some geographical variation in the relative proportions of the two alleles might be expected, the sample is not sufficiently large to test this. I see no obvious geographic pattern to the results obtained.

The systematic significance of the occurrence of Aar^1 and Aar^2 is uncertain at this time. Based on outgroup comparison, Aar^1 is a plesiotypic character state and Aar^2 is derived (apotypic). However, the taxonomic distribution of this enzyme makes it difficult to reconcile with the chromosomal phylogeny previously presented (Fig. 8b). The presence of Aar^1 in N22a (rudis) may indicate substantial gene flow from forms fixed for that allele, but it is also fixed in N22b

and appears to be present in remote populations on N21a (miamiana) and N21b (texana). Alternatively, Aar^2 may have spread throughout several forms via widespread gene flow, based on its geographic distribution. Substantially more research is required in order to come to an understanding of the taxonomic and geographic pattern of this enzyme system.

A possible variant allele was found in three colonies of N16 (all from Chaffey's Locks, Ont.). The single band obtained appeared to run a bit slower than the usual Aar^{11} band (Fig. 9b, position 4). One colony of lamellidens gave a very thick band near the position of Aar^{11} , suggesting a heterozygote of Aar^1 with a slightly slower allele (Fig. 9b, position 5). Workers from two colonies of N20 (carolinensis) appeared to be heterozygotes of Aar^2 and a rare allele that has an electrophoretic mobility midway between Aar^1 and Aar^2 , but the resolution was rather poor (Fig. 9b, position 6).

[Material examined for AAT (at least one individual reliably scored per colony, number of colonies in brackets): N16 (16), N17 (27), N18 (41), N19 (4), N20 (24), N21a (10), N21b (11), N22a (51), N22b (7), A221 hybrid colony, fulva (32), lamellidens (5), tennesseensis (9), treatae (8)]

Phosphoglucomutase (PGM-1,2): Phosphoglucomutase is monomeric, and the bands that stained must have been coded by at least two loci (probably exactly two, possibly 3 for N22b) in the species of Aphaenogaster utilized. Figure 9c illustrates representative banding patterns obtained on a "4" gel. The locus which codes for the slower running enzyme(s) has been designated PGM-1, and the other locus PGM-2. However, it must be recognized that PGM-1 (for example) may not be homologous across species. The results obtained give four separate species groupings, as follows.

(1) The banding patterns of fulva are unique to this species, with the bands produced by the four-allele system of PGM-2 particularly distinctive (Fig. 9c, examples in positions 1–4). These allozymes run much faster than any other PGM isozymes except for the slightly slower PGM-2 isozyme of tennesseensis. PGM-1 is fixed for almost all fulva (scored as *Pgm-1*²²), except for an extra, slower band for colony A167 (probably due to heterozygosity of *Pgm-1*² and a rare, unnamed allele at this locus, see Fig. 9c, position 3).

PGM-2 was scored on at least one worker for 31 colonies of fulva, and at least 3 individuals (some were males) for 24 colonies. Utilizing the data for the first worker scored on each colony (total of 62 genomes), the estimates of the proportions of the alleles *Pgm-2*¹, *Pgm-2*², *Pgm-2*³, and *Pgm-2*⁴, are 0.21, 0.15, 0.48, and 0.16, respectively. Simultaneous approximate 95% confidence intervals for the true proportions, using the method of Goodman (1965; also see Johnson and Kotz, 1969) are (0.11, 0.36), (0.07, 0.29), (0.33, 0.64), and (0.08, 0.31), respectively. Note that each allele constitutes more than 5% of the population. Although the data is sparse for testing for departures from Hardy-Weinberg equilibrium (10 female genotypes are possible), a test pooling homozygotes and heterozygotes as two classes gives a value for the chi-square test statistic of 0.15 (1 d.f., $p > 0.50$). Thus there is no evidence of differential survivorship between homozygotes and heterozygotes. The PGM-2 four-allele system of fulva would make an excellent set of genetic "markers" for testing hypotheses concerning mating systems, queen versus worker parentage of males, and possibly models of sperm mixing if artificial insemination could be used. The results for most colonies were consistent with the hypothesis that fulva has a single queen that mates once. Colony A151 was an exception; the three workers run were scored 3/3, 1/2, and 1/4. Two queens are required to explain this result (although not necessarily in the same nest), as at least five genomes are represented; it is quite

possible that there was a sample mixup. Nine of the other colonies had three different alleles present.

(2) Workers of tennesseensis produced a banding pattern similar to that in Figure 9c, position 3. The enzyme coded by *Pgm-2^s* is clearly slower than any of the PGM-2 allozymes of fulva. Although PGM-1 of tennesseensis has been scored the same as fulva (*Pgm-1²²*), one run gave evidence that the enzyme produced may have a slightly faster electrophoretic mobility.

(3) Members of the fulva-rudis-texana complex other than fulva generally produced a banding pattern similar to either position 6 or 7 of Figure 9c. This part of the figure is highly idealized. In some runs, it was often difficult to separate these two banding patterns, which appeared as a thin versus thick band. There was some evidence of hidden variation or heterozygosity present, but the expected heterozygotes of PGM-2 could not be differentiated with certainty. Although the isozymes of PGM-1 and PGM-2 did not separate well on a "4" gel (particularly for banding pattern 6), they did separate reasonably well using the buffer system of Varvio-Aho and Pamilo (1980). However, the allozymes of fulva and tennesseensis for PGM-2 did not separate well using this system, so it was only run once.

All colonies were essentially fixed for PGM-1 (this was scored *Pgm-1¹¹* for a diploid genotype). Two alleles appeared to be present for PGM-2, with *Pgm-2^r* coding for a slower running allozyme than *Pgm-2^s*. The banding pattern produced by the heterozygote *Pgm-2^{rs}* was probably present for some workers run, but it could not be reliably distinguished from that produced by the homozygote *Pgm-2^s*. A breakdown of the results by species can be briefly summarized as follows. *Pgm-2^{rs}* was found in all N16, all N17 (except for a couple of unusual banding patterns found on one run), and all but two colonies of N18 (picea). It was also found in most N22a (rudis) and some N20 (carolinensis) colonies. Either *Pgm-2^{rs}* or *Pgm-2^{rs}* (likely the former) was found in all colonies of N19, N21a (miamiana) and N21b (texana); probably both

were present in some colonies of N20 (carolinensis) and N22a (rudis). Two colonies of N18 (pacea) also produced $Pgm-2^{46}$ or $Pgm-2^{47}$ banding patterns. The banding pattern of two lamellidens workers (one from each of two colonies) and one treatae worker were also scored $Pgm-1^{11}$, $Pgm-2^{77}$. Two other treatae workers produced banding patterns somewhat similar to that of $Pgm-1^{11}$, $Pgm-2^{77}$.

(4) All workers run of N22b and the hybrid colony (A221) produced the banding pattern illustrated in Figure 9c, position 8. This appears to be the same as the banding pattern of position 6 with an extra, very low band. I believe that this band may represent the IDH allozyme unique to N22b. However, the IDH allozyme common to other forms did not stain on the PGM gels. Alternatively, this band may be due to the presence of a third PGM locus in this species. [Material examined other than fulva for PGM (at least one individual scored per colony, number of colonies in brackets): N16 (7), N17 (14), N18 (26), N19 (5), N20 (10), N21a (6), N21b (8), N22a (44), N22b (4), A221 hybrid colony, lamellidens (2), tennesseensis (4), treatae (3)]

Malic enzyme (ME): This enzyme ran anodally and could be stained successfully on three different buffer systems: "C", "4", and that of Varvio-Aho and Pamilo (1980). Individuals and species were usually fixed for one of three main bands. Almost all individuals run except for those of lamellidens and treatae were homozygous for Me^1 (although tennesseensis also appeared to be fixed for this allele, there was some indication that they had a variant band with similar electrophoretic mobility). Workers of treatae were homozygous for Me^2 ; the isozyme produced is slower than that produced by Me^{11} . Workers of lamellidens were homozygous for Me^3 ; the isozyme produced runs faster than that produced by Me^{11} . The band for Me^{23} was most distinctly separable from the Me^{11} band on the Varvio-Aho and Pamilo (1980) gel, but barely separable on a "4" gel. However, the separation of the Me^{22} and Me^{11} bands was more distinct

on the "C" and "4" gels. A variant band about 1 mm above the usual $Me^{1/1}$ band was noted for colonies A369 and A387 (both N22a, rudis) on both "C" and "4" gels run on 28 Aug., 1990.

The possible systematic significance of the Me^1 allele is that it might represent a possible synapomorphy for the fulva-rudis-texana complex (possibly plus tennesseensis). An assessment of this possibility will require examination of further outgroup species.

Esterases (EST-AS-D): Esterases produced an elaborate banding pattern which was not entirely consistent between runs and could not be scored with any certainty. However, it was noted that there did appear to be substantial interspecific variation, with banding patterns that were characteristic of one or more species. In particular, fulva showed very little activity, tennesseensis produced very fast running bands only, and N16 + N22b lacked a band common to the other species (excluding fulva and tennesseensis) that usually stained heavily, but stained heavily at a slower position. This enzyme system requires further investigation to be of much systematic use, but it does suggest a possible synapomorphy for N16 and N22b; this would conflict with the relationships of these two forms presented in Figure 8b. The variation indicated by the esterase staining in this study suggests that isoelectric focussing of esterases as employed by Heinze and Buschinger (1988) may be the best method for analyzing this system.

Glucokinase (GK): On the best run for this enzyme (13 July, 1990), seven distinct banding positions could be discerned: these can be coded 1 to 7, with 1 representing the fastest (most anodal) band with a base electrophoretic mobility of 100. The other bands with their relative electrophoretic mobilities (in brackets) relative to 1 are: 2 (85), 3 (82), 4 (70), 5 (52), 6 (36), and 7 (18). Glucokinase is a monomer (May et al., 1988). The banding patterns obtained are thus most reasonably explained by the presence of four loci coding for GK. The 7 banding

positions can be divided into a "top GK" group (1 – 4) and a "bottom GK" group (5-7). The best resolution was obtained in the "top GK" group. The resolution of the "bottom GK" group was rather poor and could not be scored consistently, but the resolution of the "top GK" group was much better. Almost all individuals of lamellidens, tennesseensis, N20 (carolinensis), N21a (miamiana), N21b (texana) and possibly treatae had bands 1 and 3, whereas individuals of N16, N17, N18 (picea), N19, N22a (rudis), and N22b had bands 2 and 4. One worker of N20 (from colony A177 from NJ1) had bands 2 and 4 rather than the usual bands for this form; this may indicate introgression between N20 (carolinensis) and N22a (rudis) at this site, but further research is required.

Malate dehydrogenase (MDH-1,2): On a "4" gel, the enzyme coded by the MDH-1 locus ran cathodally, and was monomorphic for most individuals except those of tennesseensis and treatae. The enzyme coded by the MDH-2 locus ran anodally; with individuals of the fulva-rudis-texana complex mainly monomorphic with a low level of polymorphism present. The tennesseensis workers had a much faster band than individuals of other species, and treatae and lamellidens also may have characteristic banding patterns (treatae was poorly resolved, but colony A267 of lamellidens produced a clear heterodimer banding pattern with the slowest band at the same position as the usual fulva-rudis-texana band).

Crozier (1977) used starch gels with a continuous pH 7.0 tris-citrate buffer system to examine malate dehydrogenase. This buffer system was not used in my research. The "4" gel that gave the best resolution in my investigations is a discontinuous tris-citrate buffer system, with gel and buffer pH's of 6.7 and 6.3, respectively. Crozier and I both obtained cathodal (his *Mdh-a*, my MDH-1) and anodal (his *Mdh-b*, my MDH-2) loci. However, he clearly resolved two codominant alleles for each locus, which I was unable to do. The failure to observe banding

patterns consistent with two alleles in the anodal locus may be a sampling artifact, since one allele was fixed in most populations Crozier sampled. My failure to observe clear evidence of two alleles in the cathodal locus is more problematical, as both were fairly common in Crozier's study. I obtained very inconsistent, poorly resolved results for MDH-1 on an S-9 gel run, which may have indicated polymorphic banding patterns. Nonetheless I was unable to obtain sufficient resolution in order to replicate and extend Crozier's observations on MDH.

Several enzymes appeared to be essentially monomorphic across all species: GPI and GAPDH stained well (rare variant alleles were noted, and tennesseensis had a slow band for GPI); XDH also stained well, but there was some unevenness in the band position suggesting hidden variation; ALD and PGK stained faintly, but appeared to have a single banding position. Poor resolution was obtained for DIA, GAM, GDH, α -GLU, LAP, PEP-GL, PEP-PAP, PGD, and SOD, but each of these enzymes showed evidence of polymorphism. Nothing at all was obtained for ACP, HBDH, LDH, PP, and G6PDH.

Undoubtedly some more enzymes could be resolved using starch gels with further experimentation, but I believe that switching methodologies is likely to be more profitable. Although polyacrylamide gel electrophoresis (used by Ward, 1980a) may be an improvement, the best technology for working with small organisms appears to be cellulose acetate electrophoresis (Hebert and Beaton, 1989). This technique can use very small enzyme quantities; it has been used on such organisms as the protozoan Trypanosoma cruzi, the mite Tetranychus urticae, Daphnia spp., and aphids and collembolans. It also has very short run times (usually 15 minutes), uses fewer buffer systems (two), and requires no pouring of gels. The gels are more expensive, but the extra cost should be more than compensated for by the convenience, savings in time, and the requirement for smaller amounts of staining chemicals.

2

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3.5. Morphological Analyses: Qualitative Results

Although the workers of most of the forms in the fulva-rudis-texana complex have a fairly distinctive habitus, the habitus differences are rather elusive to describe; the most tractable appear to be slight size and shape differences and differences in colour. I have detected nothing of significance in chaetotaxy differences to date, and the petiole and postpetiole offered so little of taxonomic value that I did not consider them worthy of illustration. A rogues' gallery of representative heads (Figs. 10, 11) and alitrunks (Figs. 12, 13) is presented. Readily noticed are some apparent differences in head shape and length of the propodeal spines, which are amenable to morphometric analysis.

One of the greatest impediments to understanding the nature of morphological differentiation in the fulva-rudis-texana complex has been a failure to appreciate how much size variation can occur within each form, and how morphological characters vary with size. For example, the primary component of "head shape" in this complex is in the relative dimensions of the head length and head width; this relationship is often expressed by the cephalic index ($CI = 100HW/HL$). In all members of this complex, the CI on average decreases as body size increases (see Fig. 23f); combined with the wide size variation in each form, it is quite easy for a smaller "broad-headed" form to have a relatively narrower head (lower CI) than a larger "narrow-headed" form, even ignoring intraspecific variation.

There are certainly sculptural differences among members of the fulva-rudis-texana complex, but as for shape, the nature of these differences is highly dependent on the size of the individuals. On average, the most heavily sculptured form is fulva; for example, the rugosity on the forecoxa of the fulva worker illustrated in Figure 12a (see also Fig. 12b, but contrast is poor here) is heavier than it is for any of the workers of the other forms illustrated (Figs. 12d–h, 13a–h). However, the relief of this sculpture decreases with decreasing body size. The heads

of the three fulva workers illustrated (Figs. 10a–c) show the reduction in cephalic sculpture as head width declines (the change in head shape is also apparent); note that the workers illustrated in Figures 10b–c are from the same colony. In fact, the sides of the alitrunk of the fulva minim are smooth and shiny, with much less sculpture than for any of the other illustrated alitrunks in Figures 11 and 12; the fulva minim also lacks rugose sculpture on the forecoxa. Note also the difference in cephalic sculpture between large (Fig. 11d) and small (Fig. 11e) workers of N21b (texana) from Arizona. As discussed in section 3.9, the outstanding feature of the head of the punctaticeps paratype (Fig. 11f) is not that it is so lightly sculptured and posteriorly pointed, but that it possesses these characteristics for such a large individual. Hence in order for sculpture to be useful in identification in this complex, it is necessary to account for its high dependency on size. I believe that there is probably little information here that is not more readily obtained from standard quantitative characters, but this requires more thorough investigation.

Although Creighton (1950) considered colour to be a highly unreliable character, it must be examined like any other character, with its reliability and limits of variability assessed. In the fulva-rudis-texana complex, some of the forms appear to be relatively constant in colour, and thus the dark brown (often appearing black to the naked eye) N17 and N18 (picea) forms are readily distinguished from the much lighter yellowish to light brown N20 (carolinensis) and N22b, and still separated fairly readily from the medium brown N16 and the more variable (light brown to a fairly dark medium brown) N22a (rudis). However, N21a (miamiana) has a wide range of colour variation correlated with geographic distribution, and N21b (texana) has an extremely wide colour range. Further details on the colour variation in each form are presented in section 3.14.

There are other qualitative differences in the workers, but they tend to be rather slight or exasperatingly inconsistent. Two such characters were used by Creighton (1950) to characterize fulva: the strong projection of the anterior edge of the mesonotum above the

pronotum, and the long propodeal spines of fulva being directed more strongly upwards rather than backwards. Both of these characters characterize many fulva workers (both are illustrated fairly well in Fig. 12b), but are not sufficiently consistent and nonoverlapping with other forms to be diagnostic on their own. Certainly the fulva minim (Fig. 12c) lacks the strong mesonotal projection, but it is also not much stronger in the largest fulva worker illustrated (Fig. 12a) than in the N16 and N17 workers illustrated (Figs. 12d–e). As noted by Crozier (1977), there is often a rather strong mesonotal projection in N18 (picea) workers. Similarly, there is a zone of overlap in the angle of the propodeal spines, particularly with the longer-spined forms, N17, N18, and N21a.

The most distinctive taxonomic character in the complex is found on the queen of fulva, as noted by Carroll (1975). The mesopleurae (mesothoracic sternite and episternite) are heavily rugose in queens of fulva (Fig. 14a), while they are largely smooth and shining in all other forms (e.g., Figs. 14b–d). Carroll (1975) also noted that males of fulva have thick propodeal spines, while "carolinensis" (N20 and N21a, I believe) rarely had propodeal spines. The propodeal armature of the males of other forms is somewhat variable; most have only small nubs, but N16 usually has thick propodeal spines somewhat like those of fulva. I have also briefly examined representative male genitalia for each form. There may be potentially useful characters here, primarily in the digitus and cuspis, but if so the differences are not glaringly obvious. As with other morphological studies on the sexuals, this constitutes another avenue for further investigation.

3.6. Multivariate Morphometrics I: Analysis of the Regular Data Set

The morphometric analyses presented here, unless otherwise noted, are based on the "regular" data set. This consists of the twelve variables measured (see section 2.4) on each of 264 workers in total for the ten forms. I consider the identity of each worker to be known with certainty. For 222 of them, their colonies were karyotyped. Another 40 of them are from colonies for which karyotype slides were made but the slides were not examined, since I was confident of the identity of the colony. Two workers of N22b are from colonies that could not be karyotyped, but I find this form generally easy to distinguish by habitus. The sample sizes for each form ranged from 5 for N19 to 67 for N22a (rudis); see Table 5 for the summary data on each form's sample size and mean, minimum and maximum observations, and coefficient of variation (CV) for each variable. The coefficient of variation is defined as the sample standard deviation divided by the sample mean, and here is expressed as a percentage by multiplying by 100%. The coefficient of variation has been considered to be a dimensionless measure of relative variability; whether or not it actually measures "relative variability", it is a statistic of which I am inordinately fond. Pooled within-class and between-class correlation matrices are presented in Table 6.

The functions derived from the regular data set were used to classify the workers in a further "test" data set of thirty observations. These workers included crinimera, punctaticeps, and huachucana paratypes, an N17 $\times$ N22b hybrid worker, and a voucher specimen from the $n = 18$ "?miamiana" colony of Crozier (1977). Results of this analysis are presented in section 3.7.

Logarithmic (base e) transformation made little difference in the overall numbers misclassified in the linear discriminant analyses (Tables 10, 11); the results were only marginally better when the data set was transformed (51 misclassified) than when it was not (55

misclassified). However, a comparison of the individual correlation matrices for three forms with good sample sizes (Tables 7–9) showed that most two-variable correlations were higher when the data set was logarithmically transformed (out of 66 correlations, higher correlations for transformed data numbered 42 for fulva, 63 for N20, and 62 for N22a). For this reason, the multivariate results discussed are based on the analysis of transformed data. For simplicity, the data set was not transformed for the two-variable scatterplots (Figs 19–24).

The results of the linear discriminant analysis on the log transformed regular data set (Table 11) shows that there is some misclassification between forms, but it is highly nonrandom. The most serious confusion is between the two dark brown forms, N17 and N18 (pacea). From a practical viewpoint, the confusion between N19 and N20 (carolinensis) is not serious, as the two forms have (as far as is known) allopatric distributions. It is not too surprising that there is some misclassification between N20 (carolinensis) and N22a (rudis), but the placement of six specimens of N22a into N17 was unexpected. The other cases where one or two specimens were misclassified were also unsurprising. The perfect classification of N21a (miamiana) is likely better than should be expected, for reasons to be discussed.

The separation of fulva from the other forms was essentially perfect. There was no misclassification of fulva workers as belonging to another form, and vice versa. The posterior probabilities indicate how complete the separation was of fulva from non-fulva. To four decimal places, the posterior probabilities of belonging to fulva were zero for all non-fulva workers and one for all fulva workers except for A303, which had posterior probabilities of 0.9981 of belonging to fulva and 0.0019 of belonging to N16.

A plot of the first two canonical variates (Fig. 15) from the canonical variate analysis on the regular data set (with all forms included) illustrates the morphological distinctness of fulva. Both fulva and N21b (texana) can be separated from the other eight forms quite well using only

the first canonical variate (CAN1); by utilizing the second canonical variate (CAN2) as well the chances of misclassification for fulva and N21b (texana) can be reduced further. The other eight forms are plotted in what appears to be a rather amorphous mass between CAN1 values of -4 and 5 ; however, there is some pattern here as well. For example, all N21a workers have $CAN2 > 0$, while all N16 and N22b have $CAN2 < 0$. Note that the N21a (miamiana) observation at $CAN2 = 7.65$ is a properly measured individual from colony A096 collected at FL3 (Archbold Biological Station), and often appears as an outlier in the plots involving this form. The workers in this colony have unusually narrow heads and long, thin femora relative to other workers of this form in the data set.

Of the nine canonical variates generated in the analysis of all forms, the first four account for 96.9% of the among-groups variation; CAN1 alone accounts for 51.2% and CAN2 accounts for 23.9% (Table 13a). Although some groups are separated further on other canonical variates, in general it is difficult to discern this visually; the presence of fulva and N21b (texana) help to obscure the differentiation among the other forms.

To enhance the visual separation among the eight forms, I performed another canonical variate analysis on a reduced data set; this was the regular data set with fulva and N21b (texana) removed. In the plot of CAN2 versus CAN1 for this analysis (Fig. 16a), five to six fairly distinct zones can be discerned. Four of the zones are composed mainly of N21a (the most distinct zone), N20 overlapping with N19, N22a, and N17 overlapping with N18. In addition, N16 is distinct from all except N22b, which more or less fits between N16 and N22a. In the plot of CAN3 versus CAN1 (Fig. 15b) there is better separation of N16 and N22b, although one N16 observation is still quite close to some of the N22b observations. Of the seven canonical variates generated in this analysis, the first three account for 92.5% of the among-groups variation; individual breakdowns are given in Table 13b.

Separate canonical variate analyses were performed on three selected triplets of forms, each including N22a (*rudis*), since this form is the most widespread, abundant, and most likely to be confused with another form. Each analysis of three forms can generate only two canonical variates, thus the plot of CAN2 versus CAN1 illustrates all among-groups variation. The percentage of among-groups variation accounted for by each canonical variate is given in Table 14.

The plot for N16, N22a, and N22b (Fig. 17a) shows the three forms to be reasonably distinct, although there will be some inevitable misclassifications using only measurement data. The plot for N20 (*carolinensis*), N21a (*miamiana*) and N22a (Fig. 17b) shows fairly considerable overlap between N20 and N22a. The plot for N17, N18 (*picea*), and N22a (Fig. 18a) shows even greater overlap between N17 and N18, and both overlap with N22a to some extent.

The misclassifications between certain forms is inevitable with the 12 variables utilized because of insufficient phenetic distance between these forms. The most commonly used measure of distance between two multivariate means is Mahalanobis' generalized distance, which corrects for the effect of correlation among variables (Lachenbruch, 1975). The population parameter is denoted by δ^2 , which is estimated by the sample statistic D^2 . Following Baker *et al.* (1972), I have given the square root of Mahalanobis' generalized distance; that is, D , rather than the more usually reported D^2 . These values can more readily be interpreted in terms of the standard normal distribution. Let Φ denote the cumulative standard normal distribution function. Then if equal prior probabilities are used, the probability of misclassification between two groups from approximately multivariate normal populations with multivariate means separated by the distance δ^2 is approximately $\Phi(-\delta/2)$ (Lachenbruch, 1975). To obtain a probability of misclassification that is less than 0.01, δ must be at least 4.66; for less than 0.001, δ must be at least 6.19. If the D values in Table 15 are compared with the classification results in Table 11, it can be seen that

only one observation is misclassified between forms that are separated by $D \geq 4.66$. This is an observation of N21b which is classified as N19, even though their $D = 7.61$. Lachenbruch (1975) noted that this method does not work too well if the underlying distributions are nonnormal, or if the sample sizes are small; the sample sizes of N19 and N21b were 5 and 13, respectively.

For forms separated by D values of less than 4.66, the misclassification results are highly consistent with the probabilities obtained from substituting D for δ into the cumulative standard normal distribution function expression given above. The results are somewhat complicated by the fact that some forms are close to more than one other form; for example, the value of D is less than 4.66 between N22a (rudis) and six other forms. Nonetheless we can consider the results of those correctly classified or misclassified into the other form when comparing two forms, in order to obtain an empirical misclassification rate (undoubtedly biased to some degree). For example, between N20 (carolinensis) and N22a (rudis), $D = 2.63$, which gives an estimated probability of misclassification between these two forms of 0.093 (on average, about 1 in 11 specimens). For N20, 22 specimens were classified correctly and 4 specimens were misclassified as N22a; for N22a, 51 specimens were classified correctly and 5 specimens were misclassified as N20. The empirical misclassification rate is thus $9/85 = 0.106$. Similarly, between N17 and N18 (the two closest forms) we obtain an empirical misclassification rate of $20/71 = 0.282$ compared to a theoretical rate of 0.227; there is no statistically significant difference between the empirical and theoretical rates ($p > 0.10$).

Some pairs of variables are highly correlated, both within and between classes (see Table 6); these include (using an arbitrarily chosen value of 0.9) the obvious pairs of FL and TL, ISPL and SPL, and MH and WLA, as well as HL and WLA. Several other pairs of variables have high within-class correlations (although not between-class); note particularly the high within-class

correlations between WLA and each of HW, SL, EL, FL, FW, and TL. The weaker within-class correlations between WLA and the three spinal variables reflects, I believe, the highly variant development of the spines rather than a lack of size dependency. It may also reflect a more nonlinear relationship between WLA and each of the spinal variables.

Given the highly correlated nature of some pairs of variables, it seems unlikely that all should be needed in the discriminant functions. It is also of interest as to which variables are most important to the discrimination of the forms. In the stepwise discriminant analysis on all ten forms, the variables were entered in the following order: ISPL, TL, FW, HW, EL, SL, HL, SPL, SPD, MH, FL, WLA. Each variable made a statistically significant addition to the average squared canonical correlation, although only marginally so for WLA ($p = 0.0434$, compared to $p \leq 0.0001$ for entry of each of the other variables). No variables could be removed at any stage. That ISPL is entered into the model first is interesting, as it indicates that this variable is the single best discriminator (the second, third, and fourth best choices as the first variable entered were SPL, TL, and SPD, respectively). The decision of Emery (1895) to classify members of this complex on the basis of the length of the propodeal spines is thus seen to be quite reasonable (see section 1.2). It is also symptomatic of the difficulties in morphologically separating members of this complex: the single best variable for discrimination also has the highest relative variation, as measured by the coefficient of variation, when body size is ignored. In Table 5, note that the CV for ISPL is higher than the CV for any other variable, for each of the ten forms.

The entry of SPL as the eighth variable to enter the model is due to the fact that most of the information contained in this variable has already been accounted for by ISPL. Similarly, TL (the second variable entered) explains almost all information accounted for by FL (the eleventh variable entered). It makes little difference which of TL or FL is used; that TL was

slightly better may be due to easier and more precise measurement. The very late entries of MH and WLA are not surprising. Within this complex, these variables simply are measures of body size, and information on body size is contained in other variables. I consider WLA to be the single best variable for "body size" comparisons among species, and adequate within species. Both HW and HL are more easily and precisely measured than WLA as a body size variable for comparisons within species.

After all variables were entered into the model, the last step attempted to remove a variable as no longer necessary given that the other variables were in the model. The five most difficult variables to remove were (in order starting with the most difficult) EL, HW, SL, FW, and ISPL, with partial R^2 values for removal of 0.472, 0.468, 0.443, 0.409, and 0.386, respectively. This indicates which variables contain the most unique information for discriminating among groups. The appearance of ISPL in this group is somewhat surprising, since it would be expected that SPL would contain most of the information contained by ISPL, but SPL has a partial R^2 value for removal of only 0.202. In contrast, either TL or FL are fairly easy to remove from the model, given the other variable is in the model. This suggests that ISPL really is a more discriminating variable than SPL.

Although the entry of a variable into the model may have a "statistically significant" effect in further separating the group means, it may not really be necessary for purposes of discrimination. The first seven variables into the model gave an average squared correlation of 0.339, while the addition of the other five variables raised this value to only 0.393. Based on this, I ran another discriminant analysis using only the first seven variables to be entered into the model (ISPL, TL, FW, HW, EL, SL, HL). The classification results for this analysis (Table 12) show a total of 67 misclassified individuals (compared to 51 with all variables used). The discrimination of fulva, N16, and N21b (texana) was still excellent, but there is substantially

more confusion among the other forms. If more variables were used, better discrimination would be expected, but at the current stage of model development, I would recommend measuring all 12 variables to help separate the phenetically close forms. Even WLA, which could likely be deleted with little loss in discrimination, can usefully be employed in comparisons with some of the scatterplots in Figures 19–24.

The absolute value of a standardized canonical coefficient is proportional to its contribution to the canonical variate. In the canonical variate analysis of all ten forms, the largest coefficients in absolute value for the first canonical variate (CAN1) are those for HW and ISPL, and they are of the same sign (Table 13a). This sets up a strong contrast between the broad-headed, long-spined fulva and the narrow-headed, short-spined N21b (see also section 3.8), with the other forms falling in between. The coefficient for SL is of the opposite sign, as the scapes of fulva are relatively shorter than those of N21b (texana). Although a femur of fulva is relatively slightly thinner than one of N21b, it is probable that the primary contribution of FW is to separate fulva from most of the other forms, which generally have a stockier femur. The second canonical variate (CAN2) is more difficult to interpret, but forms with relatively wide heads tend to also have wide femora (fulva is an exception), short heads, and short legs. Note that EL makes little contribution until the third canonical variate (CAN3); also note that CAN3 is placing a strong contrast between head size (HW and HL both negative) and SL (positive).

When fulva and N21b (texana) are excluded from the canonical variate analysis, HW is still the most important contributor to CAN1, but now EL is the second most important contributor, although of opposite sign (Table 13b). This contrasts the small-eyed, broad-headed forms against the larger-eyed, more narrow-headed forms. The largest contributor to CAN2 is HL. Although the standardized coefficients for the three spinal measurements do not look particularly large, CAN2 is highly correlated (correlation coefficient ≥ 0.82) with each of ISPL,

SPL, and SPD in the between-groups canonical structure. This suggests that CAN2 has captured the discriminatory power of the spinal characters utilizing other, less variable, characters. As in the canonical variate analysis with all forms, CAN3 is contrasting (at least in part) the length of the scapes against head size.

The canonical variate analysis of N16, N22a (*rudis*), and N22b essentially separates N16 and N22b from N22a using the first canonical variate, and then separates N16 and N22b on the second canonical variate (Fig. 17a). The standardized canonical coefficients (Table 14a) show that CAN1 contrasts head and body size (particularly HW and WLA) against scape and femur lengths; relative to N22a, N16 and N22b have short scapes and femora for their body size and head width. The interpretation of CAN2 is not clear, although it is based heavily on HW, HL, and WLA. In a stepwise discriminant analysis, the order of entry of variables into the model was HW, SL, FL, HL, SPD, WLA. No other variables could be entered at the 15% level of significance (highest partial R^2 to enter was 0.043 for SPL) and none of the six variables entered could be removed at the 1% level of significance (lowest partial R^2 to remove was 0.133). By far the most difficult variable to remove from the model was SL, with partial R^2 to remove of 0.647 (the next greatest value was 0.223 for HW). The use of WLA as a useful variable for discrimination among these three forms is likely due to the very large size that is common among N22b workers.

Almost all of the separation in the canonical variate analysis of N17, N18 (*picea*), and N22a (*rudis*) occurs on the first canonical variate, differentiating N22a from N17 and N18 (Fig. 17b). The principal contributors to CAN1 are clearly HW, HL, and SPL (Table 14b). This contrasts the forms with relatively short, broad heads and long spines (N17 and N18) against N22a, which has on average a narrower head and shorter spines for a fixed body size. Although the separation of N17 and N18 on CAN2 is rather poor, the scapes of N17 are probably on

average longer for a fixed head width; the other high standardized coefficients (for EL, MH, and SPL) are difficult to interpret.

The separation of N21a (*miamiana*) from N20 (*carolinensis*) and N22a (*rudis*) on CAN1 (Fig. 18a) in the canonical analysis of these three forms is based largely on the relatively larger eyes of N21a (Table 14c); N21a also has relatively long narrow femora and long spines. Most of the rather poor separation of N20 and N22a is on CAN2; the tendency of N22a to have relatively longer spines and shorter femora is reflected in the values of the standardized canonical coefficients for ISPL and FL (however, TL has an opposite sign to FL!). The high values of the HW coefficients for both CAN1 and CAN2 suggest that it is being used as a size contrast variable, but this is not entirely clear. In a stepwise discriminant analysis, the order of variable entry into the model was ISPL, FW, EL, HW, SPD, SPL, MH, FL; no other variables could be entered, and none were removed at any step. The first three variables clearly contribute the most to the average squared canonical correlation: 0.394 for the first three variables, versus 0.509 for all eight variables.

Three ways that the multivariate classification might be improved would be to: (1) use additional variables, (2) use better measuring equipment, or (3) measure more ants per colony. Of the three, measuring more ants per colony is most likely to lead to improved results with this complex; recall from section 1.6 that Daly and Balling (1978) obtained better results for identifying Africanized honey bees with this approach. Better measuring equipment, particularly for positioning the specimen, would likely improve the results for individual specimens. Both EL and FW are useful variables that are very coarsely measured at a resolution of 0.01 mm. The addition of other useful variables would be great if they were available, but I see few possibilities here.

3.7. Multivariate Morphometrics II: Classification of Test Data

Results obtained from classifying the test data set using the functions derived on the regular data set (log transformed, all forms) further help to clarify our understanding of the fulva-rudis-texana complex. When the first two canonical variates are calculated on the test data set and overlaid on the regular data set plot of CAN2 on CAN1 (see Fig. 15a), some interesting results emerge (Fig. 18b). Most obvious are the points for the paratypes of huachucana and punctaticeps. Both of these are classified as N21b (texana) by the discriminant functions with posterior probability of 1.000, since N21b is the form they are closest to. However, both are quite far removed from the N21b cluster. There is no doubt that huachucana is a distinct species, and it seems likely that punctaticeps must be as well. In contrast, the data point representing the paratype of crinimera is in very close proximity to the N21b cluster (Fig. 18b, point C), thus supplying no evidence contrary to my hypothesis that crinimera is conspecific with N21b. The statuses of punctaticeps and crinimera are considered further in section 3.9.

Two individuals that were identified as N21b (texana) prior to classification with the multivariate methods also reside clearly in the N21b cluster in the plot of CAN2 on CAN1 (Fig. 18b, points A and B). One worker (AZ: Cochise Co., Miller Canyon, 5600–5800', Huachuca Mts., coll. R. R. Snelling) was classified as N21b by the discriminant functions (also see Fig. 18b, point A), but the other, from a colony I collected at Brownsville, TX, is classified as N19. This individual has $CAN3 = 3.448$, which is substantially outside of the observed N21b range of -4.358 to -0.132 , and closer to the N19 range of 1.113 to 2.663 (the range for N21a is 2.173 to 6.272).

Although all 13 workers of N21a (miamiana) in the regular data set were properly allocated, the performance of the discriminant functions in classifying 10 other workers which I believe to also be N21a was poor. Three were classified as N21a (miamiana), three as N20

(carolinensis), and four as N19. These results were not particularly surprising, as most of the test colonies came from localities outside of the range of most colonies used in the regular data set. Three of these colonies were collected at Baton Rouge, Louisiana (these were kindly given to me by Diana Wheeler) in a very wet habitat, and clearly resemble other N21a colonies from northern Florida and Georgia. However, only one of these colonies was correctly identified by the discriminant functions, the other two were classified as N20. Five of the colonies (all four classified as N19, and one as N21a) came from southern Florida: one from Orlando, two from Everglades National Park, and two from Big Pine Key. These five cluster in the region between the point for colony A096 (largest CAN2 value for N21a) and the N21b points on Figure 18b. Despite some interference from other points, it is obvious that N21a is a very heterogeneous form, with the workers from colonies from southern Florida having a substantially different morphology from those further north. These results indicate the need to expand the data sets of N21a (miamiana), N21b (texana), and N19 on a broader geographical basis. In particular, the heterogeneity of N21a merits much further work. It is highly important to consider the geographical coverage represented in the regular data set when using the multivariate functions or the key provided in section 3.13.

Ross Crozier kindly sent me three workers from the voucher series from the Alexander Bay, FL colony identified as "rudis-group (?'miamiana') in his 1977 paper, which provided the karyotype considered to be very similar to that of fulva. One of the three specimens was randomly selected for inclusion in the test data set. Although this individual was smaller (HW = 0.78 mm) than any in the regular data set (min. HW = 0.81 mm), it was clearly classified as fulva with a posterior probability of 1.0000, and is within the fulva cluster in Figure 18b. On receiving these specimens, it was clear to me that these were very small fulva minims. I had collected a colony nearby (A094 from FL4) which I had also misidentified as miamiana until I

examined their karyotype, then went back and examined a queen. Although fulva and N21a appear quite distinct in the canonical variate plots (Figs. 15, 18b), they are quite easy to misidentify where they are sympatric. They both have relatively long propodeal spines and relatively long, thin femora, and they both nest in wood in mesic habitats (see Carroll, 1975). The SL-HW relationship (Fig. 20e) is probably the easiest way to separate workers of these two forms.

James Trager kindly sent me a small colony (A380; MO: Franklin Co., Shaw Arboretum) of an Aphaenogaster which he had found nesting in prairie habitats in Missouri (see Fig. 10g). This was of great interest, as I had hypothesized that both N16 and N22b were originally prairie (or at least mid-western) forms. Unfortunately, I could not karyotype this colony, but I included one of them in the test data set; it was classified as N16 with a posterior probability of 0.9929. Since that time, he has sent me another colony with larger workers; these clearly look like N16. Although this colony was in culture for more than a year, I was unable to obtain its karyotype.

3.8. Further Morphometric Results: Two-Variable Scatterplots

Since two-variable scatterplots present the data in a more conventional and perhaps more easily understood manner, I have presented the set of 36 scattergrams in Figures 19–24; the first 29 utilize variables measured directly ("conventional" two-variable plots), while the last seven involve indices. Any conventional two-variable plot for all ten forms shows substantial overlap between forms (e.g., Figs. 19a, 20c, 22a, 22b, 22f, 23b). However, by breaking down these plots, diagnostic characters can be found for satisfactorily separating at least some pairs of forms. In other cases, although the plots may not provide diagnostic characters, they can provide further evidence on the reliability of an identification. They also show where some of the problems occur with existing taxonomic keys, and help to illustrate the nature of morphological differentiation in this complex. The plots involving indices help show why indices are not particularly useful with this complex.

In all plots (except for two involving pairs of indices) the estimated linear regression lines have been superimposed on the scatter plots for the range of the data. The purposes of these lines are threefold: (1) they illustrate the nature of interspecific variability in the two-variable relationships; (2) they help tie together the points for a particular form; (3) they serve as a base for visually estimating intraspecific variability. The lines have not been labelled or differentiated by form; in cases where it is difficult to tell which line belongs to which form, it is essentially irrelevant to know this. Otherwise it is usually quite clear in the plots involving small subsets of the ten forms as to which form each line belongs. In most cases this can easily be determined by checking the identity of the points at the ends of the regression lines.

Table 16 presents the estimates of the regression parameters and their individual standard errors for five important relationships: HL on HW, SL on HW, ISPL on WLA, FW on FL, and EL on WLA. The standard errors utilize an estimate of the variance about the regression line

based only on the data for each form, rather than a pooled estimate from all ten forms. Estimates could also have been based on pooling the mean square error (MSE) from the ANOVA tables for testing the significance of the regressions. For those favouring such an approach, and as a quick way of making statistical comparisons, the following values for the standard deviation of Y about the regression line ($s_{Y.X}$, the square root of the pooled MSE) are presented: HL on HW, 0.01874; SL on HW, 0.03124; ISPL on WLA, 0.01434; FW on FL, 0.008114; EL on WLA, 0.008417.

In a plot of head length (HL) versus head width (HW) (Fig. 19a), only the N21b (texana) data points are reasonably well separated from those of the other forms, while those of the other nine forms appear as a poorly differentiated mass. However, each species has a rather tight zone of variability about its fitted regression line, so that it can be said that the head of N21b (texana) is usually narrower than that of N20 (or any of the other forms), while the head of N20 (carolinensis) is narrower than that of N16 (Fig. 19b), for a fixed value of HL.

If only the regression lines of Figure 19a are plotted (Fig. 19c), three distinct groups can be discerned:

- (1) the most narrow-headed group consists of N21b (texana) only;
- (2) five forms (N19, N20, N21a, N22a, N22b) compose a group with moderately narrow heads, although there appears to be some variability;
- (3) four forms (fulva, N16, N17, N18) compose a group with the broadest heads.

The interspecific variability for head shape for the four broad-headed forms is negligible compared to the intraspecific variability for each form (Figs. 19d,e; if these diagrams are superimposed, the scatterplots overlap completely). Essentially the same phenomenon is seen among N19, N20, and N21a (Fig. 19f), but in a comparison of N20 and N22a (Fig. 20a), it would appear that on average the HL increases at a slower rate as HW increases (or HW increases at a faster rate as HL increases) for N22a, although there is considerable overlap for

the two forms. The regressions of N22a and N22b are very similar (Fig. 20b), but the tendency of N22b to produce very large workers compared to those of N22a is obvious.

Couplet 19 of Creighton's (1950) key separated texana (N21b) and carolinensis (N20) from miamiana, rudis, and picea (N21a, N22a, and N18, respectively, in my usage, but likely containing other forms in Creighton's usage) on the basis of head shape. Creighton considered the latter group to have "head of the largest workers (mandibles excluded) not more than one-sixth longer than broad; head of the smaller workers approximately one-fifth longer than broad" while texana and carolinensis had "head of the worker, regardless of size, approximately one-third longer than broad". This couplet cannot do what Creighton wanted it to do; although N21b does have a relatively narrower head than the other forms, the HL-HW relationship of N20 is very similar to that of several other forms.

There are some similarities in the plots of HL versus HW (Fig. 19a) and scape length (SL) versus HW (Fig. 20c), namely the distinctness of N21b and the considerable overlap of the scatterplots for the other nine forms. However, the greater interspecific variability among the regressions for the SL-HW plot offers greater taxonomic potential. For example, even though there is greater intraspecific variability about the regression lines, it seems likely that more confident taxonomic judgements would be made on the basis of the scatterplots by using SL (Fig. 20d) rather than HL (Fig. 19b) in conjunction with HW to separate N16 and N20, although there is little difference as to which scatterplot was used to differentiate N16 or N20 from N21b. The SL-HW relationship provides other useful and/or interesting taxonomic comparisons. Reasonably good separation of fulva, N21a (at least the northern dark ones), and N21b is obtained (Fig. 20e). The separation of fulva from N20 and N22a (Fig. 20f) is not as good, but it is clear that on average fulva has comparatively shorter scapes for a fixed head width. Considerable overlap between the scatterplots of fulva and N16 (Fig. 21a) and N22a and N22b (Fig. 21b) means that

the SL-HW relationship would not be very useful for separating each pair of forms when used alone, but may contribute to the multivariate discrimination methods; this may also apply to the separation of N19 and N20 (Fig. 21c). In contrast, highly coincident scatterplots of N17 and N22a (Fig. 21d) indicate that the SL-HW relationship is essentially of no value in separating these two forms. Note that N16 is fairly distinct from N22a and N22b (Fig. 21b) and to a lesser degree from N17 and N18 (Fig. 21e). In fact, if fulva can be recognized by other means (which I believe it can), the SL-HW relationship can be used to separate N16 from the other eight forms.

Stepwise discriminant analysis selected interspinal length (ISPL) as the first variable to be entered into the model (section 3.6), while I have suggested Weber's length of alitrunk (WLA) is a reasonable size variable. A plot of ISPL versus WLA (Fig. 22a) indicates that interspecific and intraspecific variability in ISPL are both large for a fixed value of WLA (it should be noted that the length of the spines appears to have a positive allometric relationship with body size, particularly for fulva). It is clear that on average fulva has the longest propodeal spines and N21b the shortest for a fixed value of WLA, but the overlap of scatterplots between fulva and other forms (particularly N17, N18, and N21a) is considerable. The separation of N21b is reasonably good, but it can be improved by using tibial length (TL) instead of WLA on the horizontal axis (Fig. 22b). This utilizes the fact that N21b has long legs, especially compared to other forms with relatively short propodeal spines.

The ISPL-WLA relationship gives excellent separation of N21b from N19, N20, and N21a, and fairly good separation of N21a from N20 and N19 (Fig. 22c). For the four forms that occur in Ontario, the darker N17 and N18 have longer spines, on average, than the lighter N16 and N22b. For a fixed value of TL, N22a on average has shorter spines than N17 and N18, but there is still considerable overlap (Fig. 22e). Good separation of N16 from the rather similarly coloured N22a (Fig. A24), and to a lesser degree from N17 and N18 (Fig. A20) is obtained.

Surprisingly useful for taxonomic purposes was the information provided by the relationship between the width and length dimensions of the femur (Fig. 22f). Most distinctive is the unusually thin femora of fulva. The tendency in the other forms is for broader headed forms to have comparatively shorter and thicker femora, but fulva falls completely out of this pattern. Hence fulva and N16, which share the same HL-HW relationship (Fig. 19d) and overlap considerably in their SL-HW relationship (Fig. 21a), can be separated easily by their relative femur dimensions (Fig. 23a). This difference can be judged subjectively quite easily; the femora of N16 make it look like this form is on steroids, in comparison to the anaemic femora of fulva. The femora of N21b (texana) are relatively thin compared to several other forms, but their outstanding feature is their great length in larger individuals (Fig. 22f).

Creighton (1950) used a comparatively larger eye size as one of the characters that would separate miamiana (N21a) from rudis and picea. He measured the eye size by the number of facets in greatest diameter, whereas I have used eye length (EL) since the facets can be difficult to count. The large eye size of N21a is fairly noticeable, but as can be seen on a plot of EL versus WLA (Fig. 23b), there is some overlap with other forms, particularly N20 (also see Fig. 23e; the two points which look like solid circles are overlapped symbols for N20 and N21a). An earlier couplet in Creighton's key had previously separated carolinensis (N20) from miamiana on the basis of a comparatively narrower head in the former, but I have shown above that there is little if any difference in the HL-HW relationship between these two forms. Undoubtedly EL (or some other measure of eye size) is a useful character for separating N21a from several other forms. For example, the EL-WLA relationship provides fairly good (although not completely unambiguous) separation of N21a from the other very dark forms, N17 and N18 (Fig. 23c), if it were ever needed. By using HW rather than WLA on the X-axis, the separation can be

improved (Fig. 23d). In a plot of EL on WLA, the separation of N21a and N21b is quite distinct (Fig. 23e).

Figures 23f–24f illustrate selected plots utilizing indices. If an index is independent of size, then a regression of the index on a size-dependent variable (WLA was used here) should result in regression lines which are parallel (within the bounds of sampling error) to the X-axis. The cephalic index (CI) (Fig. 23f) and scape index 1 (SI1) (Fig. 24a) are clearly highly correlated with size, as expected. The spinal index (SPI) also appears to be correlated with size (Fig. 24b). The eye (or ocular) index (EI) appears to be somewhat negatively correlated with size (Fig. 24c), but femur index 1 (FI1) appears to be relatively uncorrelated with size (Fig. 24d). A plot of EI versus FI1 (Fig. 24e) shows that some forms could be separated on the basis of the indices used, such as fulva, N16, and N22b from N21a using EI, and fulva from N16 and N22b using FI1. Similarly, a plot of SPI versus FI1 (Fig. 24f) shows that some separation of forms exists here as well. However, the failure of these plots to clearly distinguish fulva from all other forms is a clear example of the inferiority of this method to the multivariate approach. Furthermore, it is difficult to see what any of the indices offer in taxonomically useful information that is not more simply available from the two-variable scatterplots, especially given the close morphological similarity among the forms. For these empirical reasons I have concluded that indices are of little value in the taxonomy of the fulva-rudis-texana complex.

3.9. Notes on crinimera and punctaticeps

Although the Aphaenogaster of the southwestern United States and Mexico require substantially more work, I nonetheless believe that decisions on the status of crinimera and punctaticeps can be made with a high degree of confidence, based partly on morphometric evidence presented in section 3.7.

I have little doubt that crinimera is conspecific with N21b, which is almost certainly texana. This is surprising, since Cole (1953) described crinimera as a subspecies of huachucana, a highly distinctive species with a small offset angular lobe on the base of the antennal scape, similar to that of flemingi. McKay (1989) has clearly illustrated the difference in the base of the scapes between texana (as well as punctaticeps) and huachucana. Creighton (1950) noted several other differences between the Arizona texana (which he referred to at this time as the variety fulvescens); although several of these may not be of much significance, the much greater size of huachucana in all castes is noteworthy.

The paratypes from the holotype nest series kindly given to me by Roy Snelling all lack the distinctive lobe of huachucana; at my request, Roy verified that the rest of the type series in the Cole collection (Los Angeles County Museum of Natural History) was not mixed. Furthermore, these specimens were essentially identical in size and appearance with the specimens of N21b (texana) collected by Stefan Cover in the mountains of Arizona (AZ1, AZ2), and match some of them well in colour. The measured specimen fit comfortably with the N21b cluster in the canonical variate analysis (see section 3.7), and was identified as N21b by discriminant analysis with a posterior probability of 1.000.

Cole's (1953) description of crinimera consists primarily of a comparison to the types of huachucana, but it is not clear whether he actually saw specimens or was going only by Creighton's description; in any case, he made no comment on the antennal scapes of the workers

or queens. He makes no mention of a comparison to texana, although behaviour alone should have been sufficient to suggest this. Creighton (1934, p. 193) noted that "the insects [huachucana] are slow, one might also say clumsy, in movement even when excited, a marked contrast to the active and energetic workers of furvescens." Cole (1953, p. 83) remarked that "the workers [of crinimera] were extremely active and agile" and further that "Creighton ... reports that the workers [of huachucana] are very sluggish when disturbed. Such behavior is definitely in contrast with that of the new subspecies."

Creighton (1950, p. 153) noted that "there appear to be no records of texana from New Mexico, although the insect must certainly occur there." Cole's records of crinimera constitute the first such published records, I believe. Fortunately, Cole's type locality information is excellent (Sandoval Co.: Bandelier National Monument, campground area at 6050' elevation) and so it should be possible to eventually karyotype colonies from this area, but in the meantime I see no reason to maintain crinimera as a separate form. A formal synonymy will be published elsewhere.

In contrast, I have little doubt that punctaticeps, described by MacKay (1989) from two workers collected in south-central New Mexico (Dona Ana Co., Jornada Experimental Range), is really a distinct species. My initial doubts on reading the description were allayed by an examination of the paratype specimen (LACM collection) kindly loaned to me by Roy Snelling. In section 3.7, it is shown that this specimen is placed as far from the N21b cluster as a paratype of huachucana in a plot of canonical variate 2 on canonical variate 1. McKay had emphasized that the head had a pointed posterior border and dorsal sculpture which was mainly punctate with little rugosity (Fig. 11f). Similar conditions are found in smaller specimens of N21b (Fig. 11e), but the punctaticeps paratype is quite large (HW = 1.02 mm, HL = 1.42 mm, WLA = 1.90 mm). The length of the legs of this specimen are also outstanding, with FL = 2.23 mm, TL =

1.57 mm, compared to the maximum values in the N21b data set of FL = 2.10, TL = 1.48 mm (A433, HW = 1.06 mm, HL = 1.44 mm, WLA = 1.92 mm). The propodeal spines are unusually short (Fig. 13e). The type workers were collected in the northern Chihuahuan desert (altitude unknown), whereas N21b is usually collected in the mountains of southern New Mexico and Arizona.

Recently Stefan Cover sent me seven workers, which he believed might be punctaticeps; I think he is correct in these identifications. Four of these workers were collected by Bert Hölldobler at Rodeo, Hidalgo Co., New Mexico (July 30, 1988) in open desert at an elevation of 4200'. The other three workers were collected by Stefan at San Simon Valley, 2.5 mi. NNW Rodeo, Cochise Co., Arizona (Aug. 5, 1990) at an elevation of 4250' in grassy desert with mesquite; he noted they were "excavating hole on kangaroo-rat mound after rain".

Only one of these workers (from the Rodeo series) has a head as sharply pointed as that of the punctaticeps paratype, but there is little doubt that these workers are conspecific with punctaticeps; more critical examination including morphometric analysis, has yet to be done (obtaining the karyotype certainly will be of interest). Nonetheless it does appear that punctaticeps is a distinct species that inhabits the open desert floor in the southwestern United States and likely Mexico. Aphaenogaster punctaticeps can be considered as a member of the fulva-rudis-texana complex for now, but it does appear to be morphologically distinct from the rest of the complex. In any case, punctaticeps is a highly intriguing ant.

3.10. Analysis of an Interspecific Hybrid

Only one colony found in this study could unequivocally be identified as a hybrid of two forms. This colony was A221, collected at ON6 (Rondeau Provincial Park), where three forms, N16, N17, and N22b are known to occur sympatrically (see section 3.11). Karyotypic and electrophoretic evidence show that this colony must be a hybrid of N17 and N22b, even when the other forms in the complex are considered. Males produced by the hybrid workers showed evidence of chromosomal instability, suggesting at least some degree of reduced fertility.

The colony was collected on 17 July, 1988, nesting under an old piece of concrete, in a narrow grassy strip with light shade between a road and the forest (see section 3.11). This grassy strip is a preferred nesting site for N22b in the park; four colonies of N22b were also collected this day in this strip, one of which (A222) was nesting under another piece of old concrete only two feet from the A221 nesting site (indeed I had questioned in my field notes whether they might belong to the same colony, but had noted that the A222 workers seemed smaller). Unfortunately, nothing particularly unusual about this colony was noted at this time; I had tentatively identified it as a "light form" colony (I was unaware at that time that three forms were in the park). Both alate queens and males were present in the nest. The nest sample was returned to the lab with nest debris and the colony fragment extracted; no nest queen was found. The primary sample was preserved at this time, rather than in the field.

The colony was reared for a considerable time before any brood was karyotyped. All karyotyped brood was male, and must have been the offspring of the hybrid workers. Four slides were made, three from prepupal brains and one from pupal testes. The three prepupal brain slides had stable modal counts within a slide (counts other than the modal number could be attributed to poor spreads), but not between slides: one slide had $n = 18$, while two had $n = 19$. The slide of the testes showed variability in counts which could not be accounted for by poor

spreads: one count of $n = 19$, four counts (plus one unreliable count) of $n = 20$, nine counts of $n = 21$, and one count of $n = 22$.

Two karyotypes from the slide with $n = 18$ and one from a slide with $n = 19$ are illustrated (Figs. 3e–g). An idiogram (Fig. 5, N17 $\times$ N22b hybrid) was prepared from CHROMPAC measurements on the karyotype illustrated in Figure 3e. In all karyotypes observed the largest chromosome was a metacentric (e.g., Figs. 3e–g), which appears to be homologous to chromosome #1 of N17. Chromosome #1 of the hybrid had measurements of $R_c = 11.45\%$ and $R_L = 7.21\%$, whereas corresponding values for chromosome #1 of N17 were 11.41% and 6.90% respectively. In fact, the length data for the first four chromosomes of N17 and the hybrid match quite closely (Fig. 5). Three of these four chromosomes are metacentrics, and hence could not come from N22b, but N16 (as well as other forms) also have metacentrics. However, a large metacentric constituting about 11½% of the haploid genome length seems to be a unique feature of the N17 karyotype; the N16 metacentrics are too small. This established N17 as one of the parents, but it left the other in doubt. There was some evidence of a very small acrocentric present (Figs. 3e, 5) which N22b has but N16 and N17 do not, but it was not certain if the small R_c value (2.78%) might not be an artifact due to the chromosome position. Note that a very small acrocentric is not obvious in the other karyotypes (Figs. 3f,g).

Chromosome #5 of Figure 3e (see also Fig. 5) is highly unusual, as it has a very small, almost absent, short arm. It not only does not match with any of the acrocentrics in the N17 or N22b karyotypes very well, but it also does not match very well with any of the chromosomes of the alternative spread illustrated from the same individual (Fig. 3f).

The establishment of N22b as the other parent was accomplished using electrophoresis of isocitrate dehydrogenase (IDH). As shown in section 3.4, all N22b workers examined were fixed for a unique fast allele for IDH, while most other fulva-rudis-texana complex workers were

fixed for a slow allele, in all cases the fast allele being absent. An N17 $\times$ N22b hybrid worker would thus be expected to have both alleles, and since IDH is a dimer, a three-band heterodimer staining pattern might be expected. Two hybrid workers run on separate occasions stained darkly at both the slow and fast positions, had no intermediate band, but also had an additional, light, very fast band (Fig. 4a). I suggest that this band is a result of the failure of the heterodimer to form properly, probably with a piece of one of the subunits breaking off during formation. It may be possible to test this hypothesis using *in vitro* subunit reassociation methods similar to that of Massaro (1967), but this has not yet been attempted. Nonetheless it seems clear to me that the fast allele could only have come from an N22b parent. Other evidence of the N22b parentage was provided by PGM and EST-AS-D; alleles that appear to be unique to the N22b form were also found in the hybrid, but these enzymes have not been satisfactorily resolved at this time to claim much support from this source.

The colour of the hybrid workers is intermediate between the light N22b and the dark brown N17. Morphologically, the hybrid workers appear more similar to N22b, particularly in the shape of the head (Fig. 11i, compare with N22b, Fig. 11h, and N17, Fig. 17e). The propodeal spines are somewhat longer than usual for N22b. The measured specimen has ISPL = 0.19, WLA = 1.78; this falls almost exactly midway between the regression lines for N17 and N22b in Figure 22d. It is rather large compared to most N17 but not for N22b. It is classified as N22a with a posterior probability of 0.6707 by the discriminant functions derived using all ten forms; I have never found N22a in Ontario, and it does not look like this form. The posterior probability of belonging to N22b was 0.3031, but only 0.001 for N17 (other non-zero posterior probabilities were 0.0168 for N16, 0.0002 for N18, and 0.090 for N20). In the canonical variate analysis on all forms, the first three canonical variates for the hybrid were CAN1 = 0.626, CAN2 = -1.424, and CAN3 = -0.567. The CAN1 value falls in the observed N17 range of

-2.346 to 1.325, and just outside the N22b range of 0.898 to 3.353. The CAN2 value falls within the observed ranges for both forms, but the CAN3 value falls outside of the observed ranges, but midway between their means (for N17, range of CAN3 is -0.155 to 2.348 and mean is 1.065; for N22b, range is -1.275 to -3.285 and mean is -2.247).

It is unfortunate that the nest queen was not collected (a return visit in an attempt to do so in 1989 was unsuccessful). The possibility exists that she might have been an N17 queen or even a hybrid herself, but I think it is more likely from the choice of nest site that she was an N22b queen. [Pearson (1983b) found that hybrid colonies produced by *Lasius alienus* queens that had mated with *L. niger* males nested within the habitat area dominated by pure *L. alienus* colonies.] If she produced the males that were in the nest at the time of collection, then they should reveal her identity (if she is pure N17 or N22b, she will produce males of only her own species). Unfortunately, at this time I am unable to distinguish the males morphologically. I have retained frozen material of this colony in an ultracold freezer, so the possibility exists of eliminating one of the possible identities of the queen by examining the worker mitochondrial DNA.

What this colony does reveal is that at least two of the forms are capable of hybridizing, and these forms have quite different karyotypes. Hybrid alate queens can be produced, as can adult males from hybrid workers. These males can probably produce sperm, but the viability of this sperm is uncertain, as it is fairly variable in chromosome number. From the karyotypes seen, most of the larger N17 chromosomes appear to be present, but the chromosomal contribution of N22b is impossible to discern. The number of slides examined is not sufficiently large to establish firmly that the retention of the large N17 metacentric is nonrandom ($p = 0.0625$), but this possibility is certainly suggested.

In any case we might expect that it would be easier for a hybrid male's sperm to produce viable backcross offspring more easily than it is for F_1 offspring to form, but we cannot be sure of this. Haplodiploidy may be a complicating factor in this. Males function quite well with one set of chromosomes, so we do not know to what degree both homologous chromosomes in the diploid set are required for proper female development, and there is even evidence (see Jessen and Klinkicht, 1990) of the female genome dominating in development. Imai *et al.* (1988a) have recorded monosomy as a chromosomal mutation occurring in ant karyotypes (see section 1.4), but it does not appear to happen very often.

White (1973) noted that almost complete pairing of chromosomes can occur in species where parent species differ by a Robertsonian change. In the fulva-rudis-texana complex, where the primary mode of karyotype evolution appears to be by Robertsonian changes, it is thus not surprising that some hybrids could be formed between some forms. It would seem particularly easy for the two $n = 22$ forms to hybridize with other forms, since their karyotypes, by essentially having each arm as a separate chromosome, should be able to pair fairly easily with any of the other karyotypes (probably excluding fulva), provided other rearrangements that cannot be detected (e.g., paracentric inversions) are not common in this complex. Nonetheless, colony A221 was the only hybrid colony detected in this study. The possibility exists that some colonies that could not be karyotyped may have been hybrids, but nothing in their morphological habitus supports this hypothesis; colonies that I could not karyotype (once I got the technique down) usually had too small of a colony fragment (especially brood) collected initially.

Barton and Hewitt (1985, p. 113) discussed two contrasting views of species: "as a set of populations delimited by genetic barriers to gene exchange; and as a set of populations maintained in a particular stable equilibrium by selection." They adopt the definition of Key (1981), stating that no fertile hybrids can be formed if two populations are to be considered as

belonging to separate biological species. Key's (1981, p. 455) definition is worded somewhat differently: "Reproductive isolation is the relationship between two populations that do not hybridize in the field, although in contact with each other, or, if they do, whose F_1 hybrids leave no progeny of reproductive age, i.e., are infertile inter se and in every backcross."

Key emphasized the problem of trying to define what constituted limited gene flow, and thought that a precise definition could only be obtained if no gene flow whatsoever was allowed. However, it seems to me that Key's definition goes beyond that point. If hybrids can produce offspring of reproductive age, but these offspring never reproduce, there will still be no gene flow between populations. In this case, is the biological species concept violated?

Haplodiploidy offers an unusual situation, since the haploid individuals are produced parthenogenetically. Consider a queen ant of species A that mates interspecifically with a male of species B. She successfully produces hybrid workers and alate queens, but any males she produces are not hybrids but are pure species A. The hybrid queens may mate but are incapable of founding a nest, and hence satisfy Key's definition on their behalf. [The scenario of Wing (1968) is a good example of this. Colonies of Acanthomyops are founded by temporary social parasitism on colonies of Lasius. He hypothesizes that hybrid Acanthomyops latipes $\times$ claviger queens may be unable to properly enact the complex behavioural responses necessary to successfully penetrate a Lasius colony.] However, the hybrid workers can produce males which reach adulthood, but for whatever reason (perhaps they are incapable of mating properly, for example) can never successfully pass on any of their genes. In this case there will still be absolutely no gene flow between populations. In this case, I believe that a strict biological species concept is not violated, as it would be if hybrid queens were able to backcross and found colonies, even though hybrid workers were able to produce "progeny of reproductive age". In a sense, the males are extended gametes of the hybrid workers, but these gametes are

reproductively nonviable. Crozier (1985) has pointed out that in a haplodiploid system (with males haploid) F_1 males do not exist in interspecific crosses, and F_2 generations are strictly not possible since all interspecific hybridizations must progress by back-crosses. It is in what is sometimes called the F_2 generation (e.g., Jessen and Klinkicht, 1990) that the first opportunity arises to observe nonviability in a male hybrid.

Documented cases interpreted as interspecific hybridization phenomena among ants are relatively scarce, and a few of these are worth considering in the context of the status of the forms in the fulva-rudis-texana complex. Additional cases to those presented here are reviewed by Pearson (1983b).

An extensive zone of hybridization between the imported fire ants Solenopsis invicta and S. richteri was studied by Ross *et al.* (1987a) using electrophoresis and gas chromatographic analysis of hydrocarbons and venom alkaloids. They identified 44 out of the 50 colonies sampled as being of hybrid origin. Evidence indicated that most hybrid colonies are not founded by F_1 generation hybrid queens. They conclude (p. 288) that "the genetic evidence for F_1 viability and random mating indicate that this zone represents a 'true hybrid zone' ... rather than a zone of overlap with some hybridization". Hung and Vinson (1977) presented convincing evidence, based on NAD-malate dehydrogenase isozymes, of a natural population near College Station, Texas, containing hybrids of the native fire ants Solenopsis geminata and S. invicta.

Ward (1980a,b) found some evidence of limited gene exchange between the closely related species Rhytidoponera impressa and R. chalybaea. An esterase allele that occurs in moderate frequencies in impressa populations was absent in all chalybaea populations except at the one locality adjacent to an impressa population. This hypothesis was supported by evidence in the colour patterns.

Jessen and Klinkicht (1990) performed extensive interspecific cross-breeding experiments using five intranidally mating species of the socially parasitic myrmicine ant genus Epimyrma. Ten types of crosses were attempted, of which eight successfully produced hybrid F₁ generation reproductive queens; the hybrid nature of the queens was confirmed by morphological examination and by isoelectric focusing of esterases. The failure of two crosses (adlerzi ♀ × corsica ♂, kraussei ♀ × corsica ♂) could be attributed to the small size of the corsica male. Jessen and Klinkicht (1990, p. 278) stated that the males "tried to mate with the heterospecific females but possibly were too small to give tactile signals with their antennae on top of the female's head and simultaneously insert the genital apparatus into the female's genitalia." The reciprocal crosses (corsica ♀ × adlerzi ♂, corsica ♀ × kraussei ♂) were successful. The mating success of F₁ hybrid females mated with nonhybrid brothers was as high as for conspecific matings, and brood production was high. However, there was a remarkable breakdown in the F₂ generation: worker production was low, half the colonies produced no males, and in colonies that did produce males, the males showed greatly reduced mating activity and mating success. Note that these males are generationally at the same stage of hybridization as the worker-produced males of the N17 × N22b hybrid colony.

Pearson (1982, 1983a,b, 1987) has conducted an extensive study of natural hybridization in England between Lasius alienus queens and L. niger males, and Wing (1968) found morphological evidence of hybridization among five pairs of species in Acanthomyops. These cases are discussed more extensively in section 3.15, but what is of interest here is that neither hybrid males nor nests founded by hybrid queens have been found in these cases, and thus the species definition of Key (1981) has not been proven violated.

By the criteria of Key (1981) and Barton and Hewitt (1985), the evidence of Ross *et al.* (1987a) is sufficient to prove that Solenopsis invicta and S. richteri are conspecific. Similarly

the decision of Ward (1980b) to maintain Rhytidoponera impressa and R. chalybaea as separate species does not meet their criteria for separate species status. However, few other ant groups have ever been analyzed on a population basis electrophoretically or utilizing other genetic techniques, and it would not surprise me if some degree of interspecific hybridization with limited gene flow was found between numerous pairs of currently recognized species. At some point a practical decision must be made as to whether two populations should be recognized as distinct species. Key (1981) recognized this, noting that the performance of his definition would need to be tested by examining how much it disturbs the existing nomenclature, particularly by a "massive regrading of taxa in one direction — i.e., species to races, or races to species". Nonetheless I would suggest that the production of males by F₁ hybrid females in haplodiploid (male haploid) organisms does not constitute much more proof than the existence of the F₁ hybrid females themselves that there is gene flow between the populations. Thus I do not consider the discovery of the N17 × N22b hybrid colony as sufficient grounds not to consider N17 and N22b (and indeed the other forms in the complex) as distinct species, in light of the other evidence presented, and I regard this colony as an interspecific hybrid.

3.11. Sympatry in the fulva-rudis-texana Complex

The sympatric occurrence of two or more forms, with little evidence of hybridization (other than the N17 $\times$ N22b colony discussed in section 3.10) or gene flow, constitutes the most powerful evidence that the genetically delineated forms recognized herein are, in fact, separate species. The cases of sympatry found in this study, where two forms were found within 500m and the identity of both forms were verified using karyotyping and (for N22b) electrophoresis, are listed in Table 17. The following additional cases of probable sympatry or less stringently defined sympatry were found in my study. At Bastian, Virginia (Bland Co.) I collected two colonies each of fulva and what I am confident is N18 (picea), although none of these colonies were karyotyped. A colony of N21a (miamiana) from FL6 was collected only 13.5 km from the only colony of N20 (carolinensis) (from FL5, also fulva here) I collected in Florida. In addition to the colonies of N19 and N22a that James Trager sent me and that I karyotyped, he also sent me a colony that he noted was very different from the other Aphaenogaster. Unfortunately, the colony died on arrival, but I am confident from the morphological habitus that this was N22b.

Crozier's (1977) finding of both N18 (picea) and N22a (rudis) at a site near the "refreshment kiosk" at Black Rock Mountain State Park, Georgia (see section 1.3) also qualifies as a case of "clear" sympatry. I was anxious to verify the microhabitat separation of N18 and N22a at this site, and so I made a visit to this park (locality GA3). The only "refreshment kiosk" I could find was a roofed open structure with a Coke machine; a park official confirmed that this was the only "refreshment kiosk" in the park. Here I collected six colonies in rotten wood (where Crozier found an $n = 18$ form, which I believe is N18) and two colonies under stones (where Crozier found an $n = 22$ form, which I am confident is N22a), before I had to terminate my collecting. All six of the rotten wood colonies and one of the colonies found under a stone were karyotyped and found to be N18 (the unkaryotyped colony was also N18, I believe).

Several possibilities exist to account for my failure to confirm Crozier's observations. Although I overturned a large number of rocks, colonies were very scarce in this microhabitat. Since it was early in the year (25 May, 1989) and reasonably cool, it is possible that the N22a colonies had not yet come up to the undersurface of the rocks; I believe that the N18 form has the highest cold tolerance of any fulva-rudis-texana complex form and thus the two colonies collected under stones may represent a small proportion (but the only active proportion on that day) of the Aphaenogaster fauna utilizing the "under stone" microhabitat. It is also possible that the altitudinal zone of sympatry has shifted somewhat, possibly due to a change in local climatic conditions since Crozier's study. Since N18 tends to occupy higher, cooler elevations than N22a, it could be hypothesized that the nature of the climatic change has been some sort of shift towards lower temperatures. Perhaps the most likely explanation is that my collecting site was not the same as Crozier's; either the "refreshment kiosk" no longer exists or someone had the unmitigated gall to move the Coke machine.

Sites of sympatry of N18 (picea) and (rudis) N22a should exist throughout the Appalachians. It would be interesting to study the altitudinal width of the zone of sympatry, and to test the hypothesis that the mean altitude of the zone increases as latitude decreases. Some evidence from the literature on the the former point is discussed in section 3.14 under Aphaenogaster picea.

The sympatric occurrence of fulva with at least one other form was found at numerous stations (Table 17), as would be expected from fulva's wide distribution. The sympatric occurrence of fulva and the widespread N22a (rudis) was found in six states at nine sites, while fulva and N20 (carolinensis) were found together in four states at six sites. There is no a priori reason to believe that any of the four forms (N16, N17, N21b, N22b) not yet found in sympatry with fulva will not eventually be found. Sites within 100 km of a collection site of fulva were

found for N16 in New Jersey, N17 in Ohio, and N22b in Missouri. Among the nine non-fulva forms, it is likely that several pairs of forms will not be found sympatrically. For example, I doubt that N16, N17, N18, or N22b will ever be found sympatrically with N21a (miamiana), or N18 (picea) with N19, N20 (carolinensis), or N21b (texana).

It is informative to examine the interaction between the forms at some of these sites of sympatry. One of the three sites where three forms were found was NJ1 (MO1 and ON6 were the other two), Bass River State Forest in the New Jersey pine barrens. Here there was a very open, dry area of pine-oak scrub, with a sharp transition to a cedar swamp. In a brief visit in 1987 I had noted both light and dark forms, but only one of the four colonies collected was successfully karyotyped. On a return visit (3 May, 1988), I collected 14 colonies (A169–A182). Seven of the colonies were collected in or at the edge of the cedar swamp nesting in or closely associated with old (probably cedar) stumps; of these, two of the colonies were identified as fulva in the field (both verified by karyotyping). Of the other five colonies from the cedar swamp, four were successfully karyotyped as N22a (rudis) (the other colony, A171, also appears to be this form). Of the seven colonies collected in the open pine-oak scrub, the six successfully karyotyped were N20 (carolinensis) (the other, A176, also appears to be this form); these were nesting in and under pieces of wood, mainly very rotten pine lying on or partially buried in the sand. Workers of N22a (rudis) at this site were distinctly darker than those of N20 (carolinensis).

Another site where three forms were found is Rondeau Provincial Park, Ontario (ON6). This park consists of a sand spit of 48 km² (Stradiotto and Stradiotto, 1984) which protrudes southward into Lake Erie. The park is noted for its Carolinian forest, which along with closely associated wetland occupies most of the spit. However, a road passes down the east side of the spit, separated from the water by a long line of cottages with reasonably well manicured lawns.

Although stones lying on the surface of the sand are rare in most of the park, many of the cottagers have ringed their lawns and driveways with medium-sized rocks; these stones are excellent ant habitats that can be examined at appropriate times. Most of the eight colonies karyotyped of N17 were found nesting in rotten wood in the forest, but one (A419) was found under a rock near a cottage, under a large spruce tree. The N22b form (six colonies collected) seems to nest primarily along a narrow grassy strip with light shade between the road and the forest, in soil with or without a covering object or in rotten wood. Cheese baits placed here to attract N22b workers resulted in some one-sided battles between N17 workers and the larger N22b workers. The single colony (A058) of N16 karyotyped from this site was found under a stone ringing a cottager's driveway. I believe that future collections of N16 at this park will be found primarily in the most open sites.

Pinery Provincial Park, Ontario (ON5) is located on the east shore of Lake Huron, just south of Grand Bend. A substantial portion of the park consists of rolling sand dunes and pine-oak savannah, as well as more mature forests of pine and oak. This is the only place in Ontario where I have found treatae, and a very dense population of tennessensis also is found here. The lighter N16 nests in more open habitats than is usual for the darker N17, although there is a range of lightly shaded environments that either may be found in. However, I have never found N17 nesting in an open area with no shade, which is not unusual for N16 (e.g., colony A409). The preference for N17 is to nest in forest with moderate to heavy shade; N16 appears to be essentially absent from this habitat at this locality. However, at OH2 (a site in Wayne National Forest, Ohio), I did find a single colony of N16 (A404) in a well-shaded, rather damp woods, in addition to four colonies of N17 (A400–A403).

In an attempt to detect either the very similar (to N17) N18 form or chromosomal evidence of hybridization between N16 and N17, 14 "dark form" colonies from Pinery Provincial

Park were karyotyped, some of which were chosen because of their relatively open nesting site or a slightly lighter colour of worker. However, all clearly had the N17 karyotype.

At ON1 (Chaffey's Locks, Ont.) both light and dark forms occur sympatrically, and as at Pinery Provincial Park the light form is N16 and exhibits a clear preference for more open habitats than the dark form. However, the dark form here is N18 (picea); more thorough sampling will be required to determine if N17 occurs here as well. At ON3 (15 km E. Gananoque), both N17 (one colony karyotyped) and N18 (three colonies karyotyped) have been found; this site is only 30 km from ON1. Throughout southern Ontario "light" (N16, except at ON6, where N22b is also found) and "dark" forms can be found sympatrically; the "dark" forms can be either N17 or N18. I hypothesize that with more extensive sampling, the distributions of N17 and N18 will prove to be parapatric, with the range of N17 occurring to the west of the range of N18.

The woods at KY2 (Daniel Boone National Forest at Morehead) was mainly mixed deciduous with the occasional large pine and with a rather open canopy. Here both "dark" and "light" forms could be distinguished in the field, but in this case the "light" form was N22a (rudis) and the dark form was N17 (recall that at NJ1 N22a was the "dark" form). All eight colonies collected were karyotyped; the results were concordant with the field colour observations (five N17, three N22a) although for one N17 colony (A350), its identification as a "dark" form colony was considered questionable at the time of collection. No microhabitat differentiation was noted; all of the colonies were found in rotten wood or in soil or leaf litter closely associated with wood. Insufficient time was spent here to examine possible differential shade tolerance, but this may be difficult to establish in any case. Although N17 tends to prefer shaded nesting sites, throughout its range N22a nests in conditions ranging from quite open sites to closed canopy forest.

The four colonies (A183–A186) obtained at NJ5 were all collected from under stones in a deciduous (mainly maple) woods. At the time of collection, I thought all colonies were conspecific, and so the discovery, upon karyotyping them, that one colony was N16 and the rest N22a (rudis) was unexpected. As these two forms are very similar in colour, they would be very difficult to differentiate in the field. A more extensive survey would be required to determine if they have microhabitat preferences at this site.

The forms N22a (rudis) and N22b have been found sympatrically at sites over a broad area (IN3, MD3, MO1). The recognition of a mature colony of N22b in the field is generally fairly easy, due to the large size of the workers and a rather distinctive gait and behavioural habitus. The interaction of these two forms merits further detailed investigation.

3.12. Application of Names

There is some risk in assigning names to the genetically delineated forms (putative species) in the fulva-rudis-texana complex without an examination of all available type material. A subsequent "type tour" of various museums will hopefully verify my conclusions, and constitutes part of my long range plans for further systematic studies in Aphaenogaster. However, I think that an assignment of names can be made with a high degree of confidence.

What I have called fulva is undoubtedly the same species generally recognized by North American myrmecologists under that name. Confirmation that this is the species described by Roger simply requires an examination of his types, if they still exist. I have no doubt that Enzmann's rubida was properly synonymized under fulva by Creighton (1950).

I have examined Wheeler's types of carolinensis in the MCZ. These are typical small specimens of the N20 form, and I believe that the name carolinensis can correctly be applied to the N20 form. There is a remote possibility that Emery's pusilla (which has priority over carolinensis) might belong to the N20 form. However, there are other possibilities for placement of pusilla, with N22a being most likely. Examination of the types of pusilla is the only way to determine if this form is identifiable. Nonetheless I consider it best to use the name carolinensis for N20 unless it can be established that pusilla is the senior synonym.

I have examined Wheeler's types of miamiana in the MCZ. These specimens are very similar to, and seem to be conspecific with, the N21a colonies from Archbold Biological Station. However, miamiana may not be the oldest available name for N21a, as Menozzi's silvestrii predates it. Creighton's (1950) synonymization of silvestrii under texana is almost certainly incorrect; I would concur with Carroll's (1975) conclusion that there is no evidence texana ranges as far east as Florida. If, as I believe, N21a represents a single biological species (see section 3.14), then Menozzi's silvestrii must represent either N20 (in which case it falls as a junior

synonym of carolinensis) or N21a (in which case miamiana must unfortunately fall as a junior synonym of silvestrii). Although silvestrii was described from only a single queen and single worker, it is probable that examination of the types would be sufficient to assign this name to one species or the other with a high degree of confidence. In the meantime, I prefer to use the name miamiana for N21a.

The name texana clearly applies to the N21b material examined. Creighton's (1950) decision to synonymize furvenscens under texana is supported in this study. However, I believe texana has a more restricted distribution than given by Creighton (1950, 1951), and there is no evidence that it occurs in eastern North America (see section 3.14).

I believe the names rudis and picea should be applied to N22a and N18, respectively. It would not be surprising to find either type series mixed, since Emery described each of these forms from material from a wide range of localities (see Table 1). Nonetheless it should be relatively easy to designate lectotypes which belong to the correct chromosomal form, even from the type localities as restricted by Creighton (1950). This treatment conforms to Creighton's concepts of rudis and picea, except that it treats them as separate species.

This leaves four forms (N16, N17, N19, N22b) without names, and an assortment of available names (azteca, pusilla, nana, punctithorax) which are not assigned. Since the types of azteca are from Mexico, they are unlikely to represent any of these unnamed forms (but the possibility that they are conspecific with azteca should be checked). As previously mentioned, the identity of pusilla requires the examination of the types; there is a possibility they might represent N16 or N22b. Wheeler's nana is likely a synonym of either carolinensis or miamiana. The types of punctithorax seem to be misplaced; Roy Snelling (pers. comm.) could not find them in the Cole collection in the LACM and Stefan Cover (pers. comm.) could not find them in the MCZ. Nonetheless I suspect that punctithorax was described from minim workers from a very

young colony of N18, and hence punctithorax would fall as a junior synonym of picea. Cole's error was in comparing his specimens only with texana, and not noticing how narrow the head of picea minimis can be.

I consider it likely that N16, N17, N19, and N22b all represent undescribed species. It is likely that the N17 form has also been identified as picea in the past, since it is almost indistinguishable from the N18 form in colour and morphology. I have some reservations on whether N17 and N18 constitute separate species, but believe it is best to treat them as such at the present time.

3.13. A Key to the Workers of the fulva-rudis-texana Complex

The following key is probably one of the most difficult to use that was ever constructed, at least for such a small group of species, as it utilizes canonical variates, karyotypes, and electrophoretic information. The electrophoretic information is the least important, as it is only necessary to provide a certain diagnosis for N22b. As much as possible, I have tried to provide alternative morphological or distributional characters in order to work around the necessity of a karyotype, but in order to separate several of the species only the karyotype is diagnostic. Information on karyotypes include the basic haploid chromosome number, but it should be remembered that supernumeraries may exist; the additional chromosome information should be sufficient to provide a karyotypic diagnosis anyhow. Although this is primarily a key to workers, the first part of the first couplet utilizes the sculpture of the queen's mesopleurae (mesothoracic sternite plus episternite), as this is by far the best morphological character found in this complex; all other morphological comparisons are for workers. The distributional data must be interpreted with care, as the sampling to date is far from complete.

For comparisons based on canonical variates, all 12 variables must be measured as defined in section 2.4. The natural logarithms of these values are multiplied by the appropriate set of corresponding raw canonical coefficients in Tables 13 and 14; these products are then added up to obtain the canonical variate value.

This key should be used when couplet 18 of Creighton's (1950; p. 140) key to the species of Aphaenogaster is reached. I am assuming that Creighton's key will work to this point, but I have not yet checked it out thoroughly. In particular, his couplet 7, which separates several western species on the basis of shorter scapes on the larger workers, needs to be reexamined. However, for the geographic range covered in my research to date, the following characters will be sufficient to identify a worker of the fulva-rudis-texana complex (adapted from Creighton,

1950): antennal scape without a basal lobe, outer face of the frontal lobe without a toothed flange, propodeum armed with distinct teeth or spines, postpetiole as long as broad or longer than broad, and any basal striae on first gastric segment which emanate from the attachment of the postpetiole limited to the basal eighth of the segment. This diagnosis will also include punctaticeps. I have not included this species in the key as it is known from so few specimens, but it does appear to be morphologically and ecologically distinct from all other forms (see section 3.9). In any case it will not affect the identification of specimens from eastern North America, which is the primary area for which this key is intended. The tentatively assigned names are used wherever possible, with form codes also given; only the form code is given for putatively undescribed species.

Key to the Workers of the fulva-rudis-texana complex in Aphaenogaster

1. Queen with mesopleurae entirely rugose (Fig. 14a). Worker with scapes relatively short for HW (Figs. 20c,e,f) and hind femora relatively long and thin (Figs. 22f, 23a); sculpture generally very heavy on medium-sized and larger workers, the forecoxa often rugose (Figs. 12a,b); propodeal spines relatively long (Figs. 22a,b), and often directed upwards; mesonotum often with anterior edge rising abruptly above the adjacent portion of the pronotum (Fig. 12b; mainly in medium-sized and larger workers); $CAN1 < -3$ and $CAN2 > 0$, using coefficients of Table 13a (Fig. 15a); karyotype of $n = 18$, of which none of the six largest chromosomes are acrocentrics with very small short arms (Fig. 1d) [widespread and abundant in eastern North America] fulva

- 1'. Queen with mesopleurae largely smooth and shining (e.g., Figs. 14b-d). Worker with scapes relatively long for HW or hind femora relatively short and thick; sculpture variable, but not as heavy as found on many fulva workers, the forecoxa with little sculpture (Figs. 12d-h, 13a-h); propodeal spines often relatively shorter, and often directed backwards; mesonotum only occasionally (primarily in N17 and picea) with anterior edge rising abruptly above the adjacent portion of the pronotum, and never as strongly as found in many fulva; $CAN1 > -3$ or $CAN2 < 0$, using coefficients of Table 13a; karyotype variable, but if $n = 18$, at least one of the six largest chromosomes are acrocentrics with very small short arms (usually two of the largest three, Fig. 1c) 2
2. (1') Propodeal spines relatively short and legs relatively long, such that values for ISPL and TL fall in the texana (N21b) zone (Fig. 22b); $CAN1 > 5$ (and $CAN2 > 2$) using the coefficients of Table 13a (Fig. 15a); karyotype of $n = 21$, with no large metacentrics and no very small acrocentric present (Fig. 2b); range includes Texas to the mountains of southern Arizona but excludes eastern North America texana (N21b)
- 2'. Propodeal spines relatively longer or legs relatively shorter, such that values for ISPL and TL fall outside the texana (N21b) zone (Fig. 22b); $CAN1 < 5$ using the coefficients of Table 13a (Fig. 15a); karyotype of $n = 16-22$, with at least one large metacentric or (for $n = 22$ forms) a very small acrocentric present (Figs. 1a-c,e,f; 2a-d); range includes eastern North America but likely excludes the southwestern United States from western Texas to the mountains of southern Arizona 3

3. (2') Scapes short relative to HW (Fig. 21f, also see Figs. 21b,e); karyotype of $n = 16$, of which five of the seven largest chromosomes are metacentrics subequal in size (Fig. 1a) N16
- 3'. Scapes longer relative to HW; karyotype of $n \geq 17$, of which no more than four of the seven largest chromosomes are metacentrics (Figs. 1b,c,e,f; 2a-d) 4
4. (3') Dark brown (piceous), with eyes relatively small for HW, and occurring in northeastern North America and throughout higher elevations of the Appalachians; CAN1, CAN2 falling within zone for N17 and picea (N18) on Figure 16a, using coefficients of Table 13b; karyotype of $n = 17$ or $n = 18$ (Figs. 1b,c) [These forms are most likely to be confused within their range with the medium-brown rudis (N22a), from which they can be separated in part by using the plot of ISPL on TL in Figure 22e. Better separation is obtained by calculating CAN1 from the coefficients in Table 14b, and comparing this value with the plot in Figure 17b.] 5
- 4'. Yellowish brown to medium brown, except for miamiana (N21a), which is dark brown outside of southern Florida but has relatively large eyes for HW (Fig. 23d) and occurs in southeastern United States at lower elevations (restricted to coastal plain?) outside of range of N17 and picea (N18); CAN1, CAN2 falling outside zone for N17 and picea (N18) on Figure 16a, using coefficients of Table 13b; karyotype of $n \geq 19$ (Figs. 1e,f; 2a-d) 6

5. (4) Karyotype of $n = 17$, of which one metacentric is substantially larger than the other chromosomes (Fig. 1b); range is likely mainly to the west of picea's range, and likely excludes the higher elevations of the Appalachians [The forms in this couplet essentially are indistinguishable morphologically.] N17
- 5'. Karyotype of $n = 18$, of which the largest metacentric is subequal in size with two acrocentrics (Fig. 1c); range is probably mainly to the east of N17's range, and includes the higher elevations of the Appalachians picea (N18)
6. (4') Karyotype of $n = 19-21$, of which there is one or two very large metacentrics (Figs. 1e,f; 2a); CAN1, CAN2 falling inside zone (defined approximately as $CAN1 > 1$) for N19, carolinensis (N20), and miamiana (N21a) on Figure 16a, using coefficients of Table 13b; at least one form present throughout Florida and coastal plain of the southeastern United States, but not confined there 7
- 6'. Karyotype of $n = 22$, of which none are large metacentrics (Figs. 2c,d); CAN1, CAN2 falling inside zone (defined approximately as $CAN1 < 1$) for rudis (N22a) and N22b on Figure 16a, using coefficients of Table 13b; both forms apparently absent from all of Florida and the coastal plain of the southeastern United States [The most difficult forms to separate at this couplet are carolinensis (N20) and rudis (N22a), which are found sympatrically in the piedmont of the southeastern United States. Using the coefficients of Table 14c to obtain CAN1 and CAN2 values, Figure 18a will aid in separating these two forms; as well, rudis tends to be medium brown, whereas carolinensis tends to be yellowish brown. Only the karyotypes are reliable for diagnosis, however.] 9

7. (6) Karyotype of $n = 21$, only one very large metacentric present (Fig. 2a); propodeal spines relatively long (Fig. 22e); CAN1, CAN2 falling inside zone for miamiana (N21a) on Figure 16a, using coefficients of Table 13b; dark brown (piceous) outside of southern Florida, where they are much lighter in colour miamiana (N21a)
- 7'. Karyotype of $n = 19$ or $n = 20$, two very large metacentrics present (Figs. 1e,f); propodeal spines usually relatively short (Fig. 22e); CAN1, CAN2 falling inside zone for N19 and carolinensis (N20) on Figure 16a, using coefficients of Table 13b; yellowish brown or a bit darker, but never dark brown, and probably absent from southern Florida 8
8. (7') Karyotype of $n = 20$, very small acrocentric present; known range includes the piedmont of the southeastern United States and throughout the coastal plain from southern New Jersey to Florida panhandle carolinensis (N20)
- 8' Karyotype of $n = 19$, very small acrocentric absent; known only from Missouri (probably found throughout much of the Mississippi basin) N19
9. (6') Larger workers frequently with $HW > 1.05$ mm (Fig. 21b); CAN1, CAN2 falling within N22b zone on Figure 17a, using coefficients of Table 14a; unique fast allele for isocitrate dehydrogenase present; scarce N22b
- 9'. Larger workers rarely with $HW > 1.05$ mm; CAN1, CAN2 falling within rudis (N22a) zone on Figure 17a, using coefficients of Table 14a; common slow allele for isocitrate dehydrogenase present; very common and widespread rudis (N22a)

3.14. Synopsis of the Species

This section summarizes, by species, information presented elsewhere in this thesis, as well as some other miscellaneous data and speculations. Some observations of other authors, particularly those of Carter (1962a,b), Carroll (1975), Cole (1940), and Van Pelt (1963) are considered in view of the taxonomic framework provided in this thesis. The colony summary lists the colonies karyotyped and/or used to obtain the regular data set for the morphometric analyses (nk = not karyotyped; nm = karyotyped but not mounted for morphometric analysis; nv = karyotyped but slide not verified; all other colonies karyotyped, verified, and used in morphometric analysis).

Aphaenogaster fulva

Diagnosis. Queen with mesopleurae heavily rugose (Fig. 14a) (largely smooth and shining for all other species in complex, Figs. 14b–d). Workers morphometrically distinct using discriminant or canonical variate analysis (see section 3.6). Larger workers generally heavily sculptured, including heavy rugosity on forecoxae; propodeal spines usually long and usually directed sharply upward; head relatively wide, scapes short, body stocky, but hind femora unusually thin for their length; promesonotal projection above pronotum often quite prominent. Colour of workers and queens usually reddish-brown, sometimes darker. Karyotype of $n = 18$, differing from the $n = 18$ karyotype of picea by the presence of a distinctive submetacentric and the absence of two large acrocentrics. Distinctive 4-allele system for PGM-2.

Distribution. Widespread in eastern North America, although I have not found it in southern Ontario, and it also appears to be absent from southern Florida (Carroll, 1975; Deyrup and Trager, 1986). Gregg (1963) reported fulva from Colorado; this needs to be confirmed. See Map 2.

Comments. This species has a preference for nesting in rotten logs and stumps in mesic forests. Although I collected no nests under stones, the use of this microhabitat has been noted by Carter (1962b), Cole (1940) and Van Pelt (1963). In North Carolina, Carter (1962b, p. 173) considered fulva "especially characteristic of piedmont mixed hardwood bottomland stands", but also made a few collections "in xeric communities of Virginia pine and post oak-blackjack oak in the Piedmont and longleaf pine in the sandhills." In Florida, Carroll (1975, p. 102) found nests "common in mesic and hydric hammocks, bayheads, cypress domes, and river swamps". He noted that in Florida fulva seemed to be absent from non-forested habitats, and more restricted to river swamps and similar moist habitats in central Florida. In the Tennessee portion of the Great Smoky Mountains National Park, Cole (1940, p. 51) found fulva at a maximum elevation of 3800 feet, and noted that "the species is nowhere common, but it is well distributed among favourable habitats". Cole's observations are concordant with those of Van Pelt (1963), who listed fulva as an occasional species in the elevation zones of 3500-4000 feet and 4100-4500 feet in his study of the high altitude (> 3500 feet) ant fauna of the southern Blue Ridge Province.

Carroll (1975, p. 105) believed "fulva bites harder and with greater tenacity than other Florida species of Aphaenogaster". It certainly seems that fulva is more aggressive towards humans than all other members of the fulva-rudis-texana complex. In the laboratory, it also appeared to be a more aggressive predator/scavenger.

Colony Summary. FL2: A240. FL4: A094. FL5: A244. GA2: A308-A309, A310 (nv). GA4: A303, A305. IN2: A383 (nv), A385 (nv). KY1: A339 (nv). MD1: A004, A006. MO2: A378 (nv). NC1: A277. NC4: A122. NC7: A285. NC8: A264 (nv). NC11: A126. NJ1: A170, A173. NJ3: A167-A168. NJ4: A151, A154, A158-A159. OH1: A394 (nv). SC1: A101 (nm), A102. SC2: A292-A294. SC4: A105-A107, A109, A112. TN2: A338 (nv).

Undescribed species #1 (N16)

Diagnosis. Basic karyotype $n = 16$, with five metacentrics subequal in size (supernumeraries known). Workers with stocky body, head relatively wide with comparatively short scapes (Fig. 21f), hind femora very stocky, propodeal spines of moderate length; colour medium brown, alitrunk often lighter than head and gaster. Most likely to be confused with rudis (N22a) or N22b (undescribed species), although there is reasonably good morphometric separation of the three species (Fig. 17a).

Distribution. Northeastern North America (see Map 2), and almost certainly extending to the midwestern United States.

Comments. This species has a preference for nesting in well-drained, drier, open habitats. Two colonies from Missouri that were sent to me by James Trager that could not be karyotyped almost certainly belong to this species; both were collected in remnant tall grass prairie habitat, nesting in the soil without a covering object. Unlike other members of the fulva-rudis-texana complex, this species will commonly nest in open areas without any shade. I believe that this species may have originated in the midwestern United States. The clearing of the forests in the northeast for agriculture and lumber has probably been of some benefit to the this species.

Colony Summary. NJ2: A185. OH2: A404. ON1: A201–A202, A424, A427, A429. ON2: A430. ON5: A188, A230–A231, A409, A410 (nv). ON6: A058. PA2: A361. See Map 2.

Undescribed species #2 (N17)

Diagnosis. Basic karyotype $n = 17$, with at least four distinct metacentric to submetacentric chromosomes; these include the two largest chromosomes, of which the largest is distinctly larger than the second largest. Worker usually dark brown, often appearing black to the naked eye, although some may be a bit lighter; morphologically almost indistinguishable from picea (N18).

Distribution. See Map 3. Although my locality records are limited (5 localities), I believe the distribution of this form occurs primarily to the west of the range of picea, in northcentral North America. I expect that this form will be found at least as far west as Iowa, as I suspect that it is responsible for Buren's (1944) "picea" records from that state.

Comments. Like picea, it nests primarily in mesic woods under rocks and in and under rotten logs and stumps. Behaviourally and morphologically it is almost indistinguishable from picea, and seems to be an ecological equivalent. It may be less cold tolerant than picea.

Colony Summary. KY2: A345–A347, A349–A350. OH2: A400, A401 (nv), A402. ON3: A228. ON5: A187–A189, A190–A191, A204, A207–A210, A212, A235–A237, A411 (nv). ON6: A412–A414, A416–A420.

Aphaenogaster picea (N18)

Diagnosis. Basic karyotype $n = 18$; two of the three largest chromosomes are acrocentrics. Worker dark brown, often appearing black to naked eye; morphologically almost indistinguishable from N17.

Distribution. Throughout the Appalachians, particularly at higher altitudes (see following comments), northeastern United States, and southern Ontario, and likely southern Quebec and the maritimes. In southwestern Ontario it appears to be replaced by N17. See Map 4.

Comments. This species appears to be the most cold-tolerant form in the fulva-rudis-texana complex, and is also highly moisture-tolerant (or dependent?). It is restricted to mesic forests. In the Appalachians, picea replaces rudis at higher elevations, but will also nest at lower elevations. There is a considerable overlap in the elevational range where they will both nest. Cole (1940) found picea at elevations from 1600 feet to more than 5000 feet in the Tennessee portion of the Great Smoky Mountains National Park. He considered the optimal elevation for

rudis (= fulva aquia of Cole) to fall between 1000 and 3000 feet. In his study of the high altitude (> 3500') ant fauna of the southern Blue Ridge Province, Van Pelt (1963) categorized colony abundance for 500' elevational intervals. He found picea abundant for all intervals between 3500' and 5000', common at 5100'–5500' and 5600'–6000', and rare at 6600'+. In comparison, rudis was common at 3500'–4000' and 4100'–4500' and rare at 4600'–5000'.

The range of ecological conditions under which picea will nest and its dominance in cool, moist forests is indicated by Cole's (1940) correlation of species distribution with forest-type. Whereas fulva and rudis were found in three forest-types (the same for each: mixed cove hardwoods, pine-heath, second-growth pine), picea was found in eight (mixed cove hardwoods, buckeye-basswood, hemlock ridge, old-field pine, second-growth pine, southern balsam fir, spruce, spruce-fir). The forest-types in which picea was the most abundant ant were red spruce (only three kinds of ants found here), hemlock ridge (eleven kinds here), buckeye-basswood (five kinds here, all highly moisture-tolerant), and southern balsam fir (only two kinds of ants here; Cole noted that the picea colonies were scarce and small). In the spruce-fir forest-type, six kinds of ants were found, of which picea was one of the two most common.

In North Carolina, Carter (1962b, p. 175) found that picea was "the most commonly collected ant in the cool, moist spruce-fir, spruce-fir-beech, hemlock, hemlock-hardwood, white pine, and oak-hardwood forests of mountain coves, slopes, and summits. Colonies were large and were found in decayed logs and stumps or in the soil under stones and logs." Carter also noted that "certain dark specimens of rudis which apparently key to A. rudis picea have been taken at sea level"; these are almost certainly not picea, and may be specimens of miamiana.

The quantitative analysis of Van Pelt (1963) led him to conclude that picea prefers nesting under rocks, but frequently nests in or under a log or stump, in litter, or arboreally. In contrast, Cole (1940, p. 13) considered picea to be "preeminently a log-dwelling ant", which "will,

however, nest in the soil beneath stones even when logs are in its habitat, but only as a result of population pressure." I am inclined to agree with Cole that picea may have a preference for nesting in wood. There are various biases which could have led to Van Pelt's conclusion, such a more abundant supply of rocks or the relative ease of collecting under rocks. However, there appears to be a great deal of local variability in the comparative utilization of rocks and logs as nesting sites for most forms in the fulva-rudis-texana complex, and picea nests under stones can also be quite large. Nonetheless it does not seem surprising that at the site at Black Rock Mountain State Park in Georgia where Crozier (1977) found both rudis and picea, it was picea that nested in logs and rudis under stones.

In Ontario, I have collected picea as far north as Constance Bay (30 km W Ottawa, site ON2, colonies not included in data set), but I have not yet attempted to determine its northern limit. My subjective impression is that colonies of picea in Ontario tend to be larger, and have larger workers, than colonies from the Appalachian mountains. Workers in large colonies are often highly aggressive.

Colony Summary. CT1: A251 (nv), A252–A254, A255. GA3. A318–A321, A323–A325. NC3: A330, A331 (nv), A332. NC10: A250. NY1: A366 (nv). ON1: A203. ON3: A223, A225, A229. ON4: A150. PA1: A405 (nv), A406–A407, A408 (nv). PA4: A362 (nv), A363, A364 (nv), A365 (nv). PA5: A029. WV1: A353. WV2: A355–A356. A357 (nv), A358 (nv).

Undescribed species #3 (N19)

Diagnosis. Basic karyotype $n = 19$. The two largest chromosomes are metacentrics that are much larger than the other (mainly acrocentric) chromosomes; very small acrocentric that is distinctly smaller than the other chromosomes not present (this chromosome is present in the very

similar carolinensis karyotype). Worker light brown to brownish-yellow; morphologically very similar to carolinensis.

Distribution. Known only from Missouri (see Map 4).

Comments. All five colonies of this form that were karyotyped were sent to me by James Trager, who noted their "carolinensis-like" behaviour. He has found this form under stones and in rotten wood, primarily in open woods, and particularly under stones on rocky slopes. In the laboratory, the movements of the workers are very similar to those of carolinensis.

Colony Summary. MO1: A368, A372. MO2: A375–A377.

Aphaenogaster carolinensis (N20)

Diagnosis. Basic karyotype $n = 20$ (supernumeraries known). The two largest chromosomes are metacentrics that are much larger than the other (mainly acrocentric) chromosomes, and the smallest acrocentric chromosome is distinctly smaller than the other chromosomes. Colour of worker usually brownish-yellow to yellow, sometimes light brown.

Distribution. See Map 4. This species occurs in northern Florida at least as far west as the panhandle, and throughout the piedmont and coastal plain as far north as the New Jersey pine barrens. However, its occurrence in the region between New Jersey and North Carolina has not been properly investigated.

Comments. As for most ant species, members of the fulva-rudis-texana complex show positive simple correlations between each pair of the following variables: colony age, worker size, worker number, and aggressiveness. The ergonomic reasons for this have been addressed by Hölldobler and Wilson (1990). Creighton (1950) recognized that much of the erroneous taxonomy in the fulva-rudis-texana complex was due to the failure to understand the relationship between size and shape in Aphaenogaster (see his remarks in section 1.2). However, I believe that he failed to

fully understand the relationship himself, particularly as it affects the taxonomy of carolinensis. My impression from collecting in the Carolinas is that carolinensis is quite capable of surviving in highly suboptimal habitats. In these habitats (in the piedmont and the interior coastal plain) carolinensis typically forms small colonies with small workers, and rarely forms a large, robust colony. In contrast, in the northern and coastal localities (NJ2, SC1, SC2) the colonies were often large with relatively large workers. Since the size of the colony is also correlated with worker aggressiveness, the smaller colonies give a very different impression in the field than large colonies. Where large colonies are present, small colonies will be too, but in these localities it is easy to attribute the differences in colony characteristics to the age of the colony. However, in some localities large colonies are simply not present; this is likely true of the locality (Tryon, North Carolina) where Wheeler collected the type specimens. If one considers such localities as having "pure stands" of carolinensis (as I believe Creighton did), then one would interpret carolinensis as having, on average, small workers with narrow heads, but this is an illusion. There is little difference in the head width-head length relationship between carolinensis and rudis (see Fig. 20a), and in fact they are rather difficult to separate using multivariate morphometrics (see Fig. 18a). In contrast, carolinensis and texana differ substantially both morphometrically and chromosomally; I can see no reason for continuing to treat carolinensis as a subspecies of texana.

Carter (1962b, p. 175) considered carolinensis to be "one of the most commonly collected ants in the upland forests of the North Carolina Piedmont. It is abundant in, and characteristic of closed-canopy middle-age and mature loblolly and shortleaf, oak-hickory, and post oak-blackjack oak forests. It is also prevalent in open middle-age shortleaf and loblolly, Virginia pine, and oak-hickory-beech, mixed hardwood bottomland, and bottomland mature loblolly pine stands. The bottomland collections were generally made in well-drained, sunny, and sandy

localities. Forms identified as A. rudis rudis replace carolinensis in the moist, shaded bottomland forests of the Piedmont." Although this seems to be an accurate interpretation of the habitat of carolinensis in the Piedmont region of North Carolina, I am surprised by the failure of Carter to find carolinensis in the coastal plain. For example, Carter recorded rudis but not carolinensis from Southern Pines, whereas I found carolinensis common in this area but I did not find rudis. I am inclined to believe that at least some of the carolinensis collections that Carter made have been misidentified as rudis.

Colony Summary. FL5: A246. GA1: A311–A315. NC2: A281. NC5: A274, A275 (nv). NC6: A290–A291. NC7: A284, A287 (nv). NC8: A266, A270–A273. NJ1: A177–A182. SC1: A103, A296–A300. SC2: A295. SC3: A117.

Aphaenogaster miamiana (N21a)

Diagnosis. Basic karyotype $n = 21$ (supernumerary chromosome variation suspected), of which the largest chromosome is a large metacentric which is much larger than the other (mainly acrocentric) chromosomes. Workers usually with relatively long propodeal spines (although not as long as those of fulva, on average); colour usually dark brown (often appearing black to naked eye), except in southern Florida, where workers are much lighter.

Distribution. See Map 4. Throughout Florida, including the Keys, possibly as far north in the coastal plain as North Carolina, and at least as far west as Louisiana.

Comments. I believe that the highly heterogeneous nature of miamiana in Florida was properly analyzed by Carroll (1975) under the name "carolinensis*" (see section 1.2). In the northern part of its range miamiana has a preference for very wet habitats, and tends to be very dark in colour similar to picea (N18) and N17. However, in the southern part of its range it tends to be much lighter and nests in a greater variety of habitats.

Only two "southern" miamiana colonies were karyotyped in my research (both from the Archbold Biological Station) and only one of these provided material for electrophoresis. Although the karyotypes for the "northern" and "southern" colonies appeared to be the same (hence my decision to treat them as belonging to a single species), the substantial morphological differentiation (section 3.7) and particularly the different AAT alleles (section 3.4) suggest that gene flow between them is quite restricted. Further sampling and analysis of miamiana is clearly necessary.

Colony Summary. FL1: A247–A249. FL2: A239, A241–A243. FL3: A096–A097. FL6: A238. GA4: A304, A306–A307.

Aphaenogaster texana (N21b)

Diagnosis. Basic karyotype $n = 21$; no large metacentric. Worker morphometrically distinct (Fig. 15a), with short propodeal spines, long scapes and legs, and relatively narrow head (most easily separated from other fulva-rudis-texana complex members by the interspinal length–tibial length relationship, Fig. 22b); colour variation extreme (see comments).

Distribution. The distribution of texana certainly ranges from Texas to the mountains of southern Arizona. Based on my own collecting and the more intensive sampling efforts of Carter (1962a,b) in North Carolina and Carroll (1975) in Florida, I believe that texana is absent from the southeastern United States. Further sampling in the midwest and Mexico will be necessary to discern its range in these regions. However, I believe that the record of texana from Marshall Co., Illinois (DuBois and LaBerge, 1988) is almost certainly in error (I would predict that this record represents either N19 or N22b, both undescribed).

Comments. More collections of this species are obviously necessary, since the sample obtained in this study is so obviously biased. However, the available material does present a fascinating

range of colour variation. The single colony karyotyped from Texas fits Emery's description of a "honey-yellow" colour, whereas my specimens (not karyotyped) from Brownsville, Texas are better described as being reddish-brown. These colonies are lighter than the Arizona specimens, which themselves are highly diverse. Most of the colonies I have seen have workers which fit Wheeler's (1915, p. 413) description of the colour of furvescens well: "reddish brown, with the gaster dark brown, except at the extreme base. In some specimens the head and nodes of the petiole and postpetiole are also distinctly infuscated." However, the workers in some colonies collected by Stefan Cover are quite dark, almost piceous, and appear dark brown to black with the naked eye. Stefan thought that he had noticed some microhabitat separation correlated with colour, with the colonies with darker workers nesting in more deeply shaded woods. This merits further study.

Colony Summary. AZ1: A432–A438. AZ2: A439–A442. TX1: A422. [All colonies from Arizona were verified as $n = 21$ while I was at the University of Guelph, but only A435–A436, A441–A442 have been examined using the Zeiss microscope at Carleton University.]

Aphaenogaster rudis (N22a)

Diagnosis. Basic karyotype $n = 22$ (supernumeraries known), essentially all acrocentrics; common slow allele for isocitrate dehydrogenase present. Worker intermediate in all morphometric characteristics; colour usually medium-brown.

Distribution. Map 3 presents the 23 localities at which I karyotyped one or more rudis colonies; this gives the best estimate to date of the range of this species. Undoubtedly this map underestimates the true range of rudis, particularly to the west, but some further interpretations can be suggested. It appears that rudis is an abundant ant throughout the Piedmont and lower elevations of the Appalachians, and extends westward through the forests of Tennessee,

Kentucky, and southern Ohio and Indiana at least as far west as Missouri. Gregg's (1963) records from Colorado need to be verified. Although I expect rudis to be found somewhat further north than currently recorded, I have never found this species in Ontario, and Crozier (1975) did not record it from the vicinity of Ithaca, New York. I believe that Carroll (1975) is correct that there is no evidence of rudis occurring in Florida. I further suspect that rudis is scarce or absent throughout much of the southeastern coastal plain, despite the records of Carter (1962b); see the "Comments" on Aphaenogaster carolinensis in this section.

Comments. This species nests in a wide range of moderate conditions, but seems to prefer mesic woods with some openness in the canopy. The elevational preferences of picea and rudis are discussed in this section under the former species. On a broad geographical basis it can present very different field impressions. At Cheaha State Park, Alabama (AL1, elevation approx. 2300'), all eight colonies collected (only A085 karyotyped) were fairly small colonies with small workers; my impression was that they were nesting in a rather marginal habitat. In contrast, some colonies from Kentucky and Tennessee (for example) were often large and robust, with large workers. The seven colonies collected at NJ2 were nesting in the soil under pieces of old asphalt at the side of a road in a pine-oak habitat typical of the pine barrens, whereas at NJ1, also in the pine barrens but where carolinensis was present, rudis appeared to be restricted to nesting in rotten wood in the cedar swamp area (see section 3.11). Also see the remarks on rudis in this section under carolinensis.

Colony Summary. AL1: A085. IN1: A382. IN2: A384, A386–A388 (nv). IN3: A389, A391–A392 (nv). KY1: A073, A341–A344. KY2: A348, A351–A352. MD1: A009. MD2: A359 (nv). MO1: A369–A370, A373. NC1: A278. NC2: A279–A280, A282–A283. NC4: A121, A123. NC9: A326–A328. NC11: A125. NJ1: A004, A169, A172, A174–A175. NJ2:

A160–A166. NJ4: A155–A157. NJ5: A183–A184, A186. OH1: A393, A395–A397. PA3: A017, A019. TN1: A080 TN2: A334–A337. VA1: A256–A258, A259–A260 (nv).

Undescribed species #4 (N22b)

Diagnosis. Basic karyotype $n = 22$, essentially all acrocentrics; unique fast allele for isocitrate dehydrogenase present. Worker light brown to yellowish-brown, often unusually large with a rather rectangular head.

Distribution. See Map 4. Based on the limited collecting records, this species appears to be reasonably widespread in northeastern North America and the midwestern United States, although it is only occasionally encountered.

Comments. I believe this species (and N16) may have originated in the midwestern United States. It appears to have a preference for habitats with well-drained soils (possibly preferring sand) and light shade. James Trager (pers. comm.) has found this species in Merimac State Park, Missouri, in semiopen white oak forest with a very open understory; he describes the soil as being cherty with sandstone and chert. I collected colony A390 from locality IN3 in a small prairie remnant with sparse oak-pine on pure sand. The nest site preferences of N22b at Rondeau Provincial Park, Ontario are discussed in section 3.11.

This species presents a very distinctive field impression. Mature colonies can be quite large, with very large workers that appear to have a distinctive gait. Although the head width–head length relationship of this species is similar to that of *rudis* (Fig. 20b), the head tends to be more rectangular with squarer posterior corners. There are also sculptural differences near the eye, but the consistency of this character requires further study.

Colony Summary. IN3: A390. MD2: A360. M01: A367 (nk). ON6: A218, A219 (nk), A220, A222, A415, A421.

Colony Summaries for Outgroup Species

A. lamellidens: KY1: A340. NC1: A276. NC6: A289. NC7: A286, A288. NC8: A261, A269.

A. tennesseensis: KY1: A070. ON5: A196–A198, A200, A323–A234.

A. treatae: GA1: A317. ON5: A215.

3.15. Interspecific Mating in Ants and the Evolution of Sperm Parasitism

I propose that, under certain conditions, interspecific mating by queens of the eusocial Hymenoptera with the subsequent production of infertile hybrid offspring can be selected for as part of a mixed evolutionarily stable strategy. The model will be developed under the strict species definition of Key (1981). This definition does not allow for any introgression between species; F_1 hybrids may be formed, but they leave no progeny that reach reproductive age. It is suggested that the reduction of fitness to an interspecifically mated queen caused by the production of infertile gynes (daughter queens) can be offset by the production of hybrid workers, if the hybrid workers allow the utilization of nest sites that colonies with intraspecifically mated queens cannot utilize with sufficient efficiency. Queens that mate interspecifically can be considered "sperm parasites" on the males of the other species. Documented examples of interspecific hybridization in the ant genera Lasius and Acanthomyops are reexamined in the context of the model.

Members of the eusocial Hymenoptera live in colonies, are haplodiploid, and usually have a reproductive division of labour in the female sex. With rare exceptions, males are produced from unfertilized (haploid) eggs, and females are produced from fertilized (diploid) eggs. Females are reared either as queens (reproductive females, or gynes) or one or more worker castes. In a monogynous colony, all workers are daughters of the single queen; daughters may also be reared as new gynes. Males may be the offspring of either the queen or one or more workers; the proportion of males produced by the workers varies from 0 to 1, depending on the species and sometimes on the local environment. The inclusive fitness of each member of the colony depends on maximizing the genetic output of the colony through the production of new queens and males, subject to the optimality constraints imposed by queen-worker conflict.

The lifespan of an adult queen and the colony she founds can be divided into a prereproductive stage and a reproductive stage. In the prereproductive stage the virgin queen leaves the parent nest, mates, and locates a nest site; this stage continues through the early founding and growth of the new colony. For uniformity, the reproductive stage for a given species can be defined as starting after the minimal amount of time it takes from the emergence of the adult queen to the production of the first reproductives in her new colony.

Consider a species (Species A) of ant that forms monogynous colonies, and for which the queens can mate with the males of another ant species (Species B). We will assume initially that a queen mates with a pure strategy: either entirely intraspecifically or entirely interspecifically. This is accomplished easily if the queen mates with only one male, of course, but it is also likely if the queen mates with several males in a "swarm" composed mainly of males of a single species. The consequences of interspecific mating for a queen is that any daughter offspring she produces will be hybrids, and thus infertile (according to our species definition). If workers of Species A are essentially infertile anyhow, the production of hybrid workers will not necessarily result in a loss of fitness, but any gynes produced are also infertile hybrids, and thus "genetic junk". However, any sons produced are pure Species A males, and thus a queen need not suffer a complete loss in fitness by mating interspecifically.

For a defined population, let R be the event that a new queen reaches the reproductive stage, and let F be a random variable representing the lifetime inclusive fitness of the new queen. Where necessary, a subscript i will denote mating strategy, with $i = 1$ for intraspecific mating and $i = 2$ for interspecific mating. The probability of reaching the reproductive stage for a randomly chosen new queen that is about to leave the colony and mate under strategy i is $P_i(R)$, and her expected lifetime inclusive fitness is $E[F_i]$. However, the expected lifetime inclusive fitness of a queen that has reached the reproductive stage is $E[F_i|R]$; note that

$$E[F_i] = P_i(R)E[F_i|R].$$

In general $P_i(R)$ will be very small due to the high mortality rate of new queens, thus usually

$$E[F_i] \ll E[F_i|R].$$

Also define a parameter c such that

$$E[\bar{F}_2|R] = cE[F_i|R].$$

In general we would expect $c < 1$; that is, among queens that have reached the reproductive stage, an interspecifically mated queen will, on average, achieve only a fraction of the expected fitness of an intraspecifically mated queen. The most obvious reason for this is due to the production of infertile gynes, but other factors that may reduce c include possible reduced viability or ergonomic efficiency of hybrid workers, reduced alate production, and shorter expected lifetime of colonies with interspecifically mated queens. [However, it seems to be not entirely impossible that c can be ≥ 1 , under certain conditions to be discussed later.] Selection will operate on the $E[F_i]$ values rather than the $E[F_i|R]$ values, however. To compensate for the lower $E[F_i|R]$ value for colonies with interspecifically mated queens, they must have a sufficiently higher probability of getting to the reproductive stage, that is $P_2(R) > P_i(R)/c$, for selection to favour interspecific mating if it has a genetic basis. The simplest way for this to occur is if there is high intraspecific competition for "good" nest sites among intraspecifically mated queens, and there are "marginal" nest sites available which can only be successfully utilized (or utilized with sufficiently greater efficiency) by colonies with interspecifically mated queens by virtue of their hybrid workers.

For a species in which the colonies have one singly-mated queen and the queens produce the males, it has been argued that the equilibrium sex ratio of reproductives (assuming complete outbreeding in the population) in terms of energetic investment in males is $s = 0.25$ if the workers control the sex ratio or $s = 0.50$ if the queen is in control of the sex ratio (Trivers and

Hare, 1976). Evidence in ants suggests that it is usually the workers that are in control of the sex ratio (Nonacs, 1986). If a hybrid colony produced reproductives at the equilibrium sex ratio of $s = 0.25$, then 75% of its energetic investment in reproductives will have been wasted on the production of infertile gynes. If all other factors are equal, this gives a value of $c = 0.25$, which is clearly a major decrease in $E[F|R]$ for mating interspecifically. However, if the probability of an interspecifically mated queen reaching the reproductive stage is greater than four times that for an intraspecifically mated queen, then interspecific mating should be selected for if it has a genetic basis.

Clearly interspecific mating cannot be a pure strategy, as males produced by interspecifically mated queens must mate with gynes produced by intraspecifically mated queens. I propose that it can, however, exist as part of a mixed evolutionarily stable strategy (ESS), in the sense of Maynard Smith (1982), with obvious parallels to his classic Hawk-Dove game (see also Dawkins, 1989). Like hawks, a population of all interspecifically mated queens cannot exist. Like doves, a population of all intraspecifically mated queens could coexist fine, but in the same way that a hawk strategy could invade and spread in a population of doves, a strategy of interspecific mating can invade and spread in a population of intraspecifically mated queens, if there is a fitness advantage in doing so. As the proportion of queens that mate interspecifically increase, however, the competition for "marginal" sites among such queens will increase, and the competition among the intraspecifically mated queens for "good" nest sites will decrease; the function f such that

$$P_1(R) = f\{P_2(R)\}$$

is strictly monotone decreasing. We would also expect that the production of extra males in the population to affect the relative fitness value of a male compared to that of a Species A gyne. Although exactly how the relative fitness value would be affected is not clear at this time, we

would expect that the value of interspecific mating relative to intraspecific mating would be reduced as p , the proportion of the population of queens that mate intraspecifically, decreases. The value of p such that $E[F_1] = E[F_2]$ will be the equilibrium (or evolutionarily stable) proportion, p_E , that should mate intraspecifically ($q_E = 1 - p_E$ is the proportion that should mate interspecifically).

Each queen in the population will "play the mating game" only once. It does not matter what the probability of any given individual queen mating interspecifically is, it matters only that the proportion in the population that actually does is $q_E = 1 - p_E$. In practice, we would expect that Species A queens that fly near the "marginal" habitat are also more likely to encounter and mate with males of Species B than are queens flying near the centre of the "good" habitat. Thus the probability that a new queen mates interspecifically is likely to be positively correlated with the proximity of its parent colony to marginal nest sites; this increases the probability that an interspecifically mated queen is going to find the type of habitat in which it can successfully compete.

Interspecific matings on the part of the queens are "purposeful" in the sense that selection would operate against the strengthening, and for the weakening, of premating reproductive isolating barriers for queens of Species A (it should operate to strengthen them for males of Species B, although possibly not as strongly). A simple mechanism which is likely to have a genetic basis that selection could operate on is in the timing of the mating flights. It would seem that the proportion of Species A gynes that are released at the same time as the males of Species B could be easily adjusted by natural selection to try to obtain the correct p_E value for the local conditions. It also seems possible that if (as we would expect, subject to other constraints) Species B mating flights try to shift away from those of Species A, Species A might "track" those of Species B in evolutionary time for at least a proportion of the Species A gynes.

If interspecific mating can be selected for as part of a mixed ESS, interspecifically mating queens of Species A can be considered "sperm parasites" on the males of Species B, since they are reducing the fitness of the Species B males in order to increase their own fitness. For sperm parasitism to be selected for, it is obviously necessary that viable hybrid worker offspring can be formed, and these hybrid workers can help the queen rear her sons. It also seems clear that the higher the value of c is, the easier it will be for interspecific mating to evolve as part of a mixed ESS. Several factors need to be considered in evaluating the likelihood that sperm parasitism could evolve; these can be listed as follows.

1. *Genetic compatibility.* The hybridizing species must be sufficiently genetically compatible to produce hybrid workers, and thus are more likely to be closely related (although not necessarily sister species), with divergence from a common ancestor being relatively recent in evolutionary time. Phylogenetic differences in the nature and rate of genomic evolution and phylogenetic and geographical variation in patterns of speciation make it likely that sufficient genetic compatibility for hybridization will have a very patchy phylogenetic distribution (social parasitism in ants also has a very patchy phylogenetic and geographical distribution; see Hölldobler and Wilson, 1990). Any degree of nonviability of hybrid worker offspring (e.g., a higher proportion that die prior to adulthood) will reduce the value of c , although a degree of nonviability of hybrid gynes might actually increase the value of c if it causes a shift towards increased male production.

2. *Queen versus worker production of males.* The evolution of sperm parasitism is easier if the queens normally produce all of the males. The proportion of males produced by workers is a highly variable trait in eusocial Hymenoptera (Bourke, 1988). In some species of ants, the workers lack ovaries (e.g., all *Solenopsis* and *Pheidole*) and thus clearly cannot lay eggs. In many species, the workers have ovaries but male production is suppressed in the presence of the queen. However, the workers will lay eggs that will produce males after the death of the queen

(e.g., many *Aphaenogaster*); the loss of this contribution to the inclusive fitness of the colony must be considered for colonies with interspecifically mated queens. In other species, most or all of the males produced in queenright colonies are sons of workers. This clearly does not favour the evolution of sperm parasitism; with other factors held constant, it would be expected that c would decrease towards zero as the proportion of males normally produced by workers increases towards one. However, as long as the queen produces at least some males, the evolution of sperm parasitism cannot be considered impossible. A possibility that must be considered is whether the queen would be allowed to produce more sons when the hybrid workers were unable to do so.

3. Male investment strategies. The lower the value of s (the equilibrium sex ratio of investment in males) is, the higher the loss of fitness will be if colonies with interspecifically mated queens invest in males at the same proportion. Factors which appear to influence the value of s include the degree of control over s exerted by the queen (Trivers and Hare, 1976), the number of times the queen mates, with multiple mating reducing worker control of the sex ratio (Woyciechowski and Łomnicki, 1987), and the local availability of resources (scarcity favouring male production, abundance favouring gyne production; see Nonacs, 1986a,b). Despite theoretical advantages of each colony investing in each sex at the equilibrium sex ratio (see Taylor and Sauer, 1980), there is considerable variation in the actual sex ratio among colonies within most species (Nonacs, 1986a,b; Boomsma and Grafen, 1990). It obviously would be to the benefit of a colony with an interspecifically mated queen to shift its energetic investment in reproductives towards all male production. If colonies with intraspecifically mated queens were to shift towards increased gyne production, colonies producing all males will not necessarily suffer any loss of fitness. The ability of ant colonies to specialize on the production of a single sex is well documented. For example, Helms and Rissing (1990) found that all colonies of *Pheidole xephila tucsonica* and

Pheidole desertorum specialized in producing a single sex, the only mixed colonies being a few male-specialist colonies of P. desertorum that produced a small number of gynes.

The ability of a colony to shift its alate production should be influenced by how well it "knows" the mating strategy utilized by its queen. This might be probabilistically cued, for example, by the location of the colony's nesting site. Certain phenomena already noted in ants may be useful preadaptations for the evolution of sperm parasitism. Nonacs (1986b) has reviewed the evidence that gyne production is favoured when resources are abundant and male production when resources are scarce; thus, colonies in "marginal" habitats may tend to produce a higher proportion of males. Colonies also tend to specialize in males when the colony is young; this would also be beneficial to colonies with interpecifically mated queens if they have shorter expected lifespans compared to colonies with intraspecifically mated queens.

4. *Utility and ergonomic efficiency of hybrid workers.* Hybrid workers must have some sort of competitive advantage over Species A workers under some set of conditions; it is also possible that they will have to outcompete Species B workers in these conditions. I believe that the most likely place hybrid workers will have a competitive advantage is in "transition zones"; that is, habitats intermediate in critical ecological variables between the habitats preferred by Species A and B. The confounded variables of moisture and temperature may be the most important for niche separation of many pairs of closely related ant species (see Talbot, 1934). Hybrid workers may be physiologically intermediate in their moisture and/or temperature preferences. The possibility of hybrid vigour through heterosis should not be discounted (e.g., hybrids in the sunfish genus Lepomis; see Hubbs, 1955). Hybrid workers can have a lower unconditional mean ergonomic efficiency as nonhybrid workers (although this will further reduce the value of c) as long as they have a higher conditional mean ergonomic efficiency in their preferred habitat.

5. *Habitat variability.* On a broad geographical basis, the quality of the habitat for providing nest sites will vary considerably, on a continuum from optimal to unusable. The division of nest sites into "good" and "marginal" nest sites is a useful dichotomization of this continuum. Of critical importance to the evolution of sperm parasitism is the spatial and temporal distribution of "good" and "marginal" nest sites. The relative abundance of "marginal" nest sites should affect the ability of the interspecifically mated queens to find such nest sites, with rarity possibly depressing the value of $P_2(R)$ such that the "c gap" cannot be overcome. However, in some localities "marginal" nest sites may be relatively common, and perhaps even more abundant than "good" nest sites. The average quality of "good" nest sites on a local basis would also be expected to vary throughout the range of a species, which may affect both the absolute $E[F_i|R]$ values and their relative value c . Certainly the quality of nest sites would be expected to be suboptimal towards the edges of the range of a species; selection for sperm parasitism may be favoured if Species A occurs in such a locality, and Species B is more centrally located within its range at the same locality.

The temporal variability of habitat must also be considered. A substantial change to a habitat may open up nest sites for which both Species A and Species B are poorly adapted, but which hybrids can utilize. Human alteration of habitat has often been implicated in hybridization phenomena (e.g., Pearson, 1982; Wasserman, 1957; White, 1973).

6. *Intensity of intraspecific competition.* Competition in ants (both intraspecific and interspecific) has recently been reviewed by Hölldobler and Wilson (1990). Competition will take place throughout a colony's lifespan by both interference and exploitation. Janzen (1967) observed competition by direct combat in founding queens of the Acacia-inhabiting ant Pseudomyrmex ferrugineus, but most competitive actions will be undertaken by the workers. Intraspecific competition can be expected on theoretical grounds to be particularly intense in many ant species

by virtue of their trophic level in the food web and the large size and long life of their colonies (Hölldobler and Wilson, 1990). Species with large colonies also tend to be highly territorial towards conspecifics and often other species (particularly competitors) and have a propensity towards engaging in "ant wars" (also see Hölldobler and Lumsden, 1980, and Jansen, 1967). A founding queen has an extremely small chance of successfully establishing a colony within the territory of a mature conspecific colony in most species. Nest sites become available with the death of existing colonies; the turnover rate can be low due to the great longevity of mature colonies in some species. Intraspecific competition can also greatly depress the fitness of colonies which have reached the reproductive stage. Pontin (1961) showed that alate production by pairs of *Lasius flavus* colonies (in the absence of their chief interspecific competitor, *Lasius niger*) as a function of the distance between the colonies could be adequately described by a linear model passing through the origin. This will also tend to increase the evolutionary pressure to find alternative nesting sites.

What currently seems to be lacking in the literature on ant competition are any estimates of what I term *coefficients of intraspecific competition*. Two coefficients which are useful in the context of the model can be defined as follows. The *coefficient of intraspecific competition for colony establishment* (I_R) can be defined as the expected probability a new queen will reach the reproductive stage in the presence of intraspecific competition divided by the expected probability she will reach the reproductive stage in the absence of intraspecific competition. The *fitness coefficient of intraspecific competition* (I_F) can be defined as the expected lifetime fitness of a new queen in the presence of intraspecific competition divided by the same quantity in the absence of intraspecific competition. We would normally expect that both I_R and I_F would take on values between 0 and 1, with each tending to decrease as the intensity of interspecific competition increases, but there may be some levels (likely fairly low) of intraspecific competition where the

probability of successfully establishing a colony increases through activities such as brood raiding (Eldridge Adams, pers. comm.). Both values should be estimable in practice, at least on an experimental basis, although probably only with considerable effort.

7. *Availability and mate specificity of Species B males.* It is likely that Species B will be a common species, in order that there be an abundant supply of Species B males. Species B males should also not be too discriminating in whether they mate with Species B or Species A queens. Strong sexual dimorphism in Species B (this is also likely in Species A) with very small males relative to the queens may produce a numerical surplus of males in the population that will not get to mate, particularly if queens are monogamous. Thus intense competition among Species B males for Species B queens may predispose the males to being very nondiscriminating towards Species A queens.

8. *Presence of interspecific competition.* A differential in the intensity of interspecific competition between "good" and "marginal" nest sites can either enhance or decrease the likelihood of sperm parasitism evolving. This may make the utilization of "marginal" nest sites in stable ecosystems with a high degree of species packing (such as in tropical rainforests) too difficult for sperm parasitism to evolve.

Can sperm parasitism be a reciprocal phenomenon between two species? I cannot think of any reason that makes it clearly impossible in all cases, but there are several possible asymmetries which make it more likely to be a one-way phenomenon.

1. *Physical or behavioural differences in reproductives restricts interspecific matings to one-way crosses.* Jessen and Klinkicht (1990) documented this phenomenon in their cross-breeding experiments involving five species of the socially parasitic ant genus Epimyrma. Crosses of E. adlerzi ♀♀ × E. corsica ♂♂ and E. krausseii ♀♀ × E. corsica ♂♂ failed completely, although

the reciprocal crosses were very successful. They attributed the failures as likely due to the relatively small size of the *E. corsica* ♂♂, noting (p. 278) that "they tried to mate with the heterospecific ♀♀ but possibly were too small to give tactile signals with their antennae on top of the female's head and simultaneously insert the genital apparatus into the female's genitalia."

2. *Differential proportion of males produced by workers.* If Species A produces a lower proportion of males via workers than Species B, it is more likely to realize an advantage by mating interspecifically.
3. *Differential ratios of energetic investment in males.* Both the value of *s* and the ability of individual colonies of a species to shift the sex ratio of reproductives may be important factors.
4. *Differential in average worker size.* Hybrid workers may be more useful if they are larger, say, than normal workers. This possibility was suggested by Pearson (1983b) and is discussed in the synopsis of his work on *Lasius alienus* × *niger* hybrids following.
5. *Different mating times.* If Species A usually mates earlier in a season than Species B, it may be easier for it to hold some gynes in the nest for later mating with males of Species B than it would be for Species B to accelerate gyne production to mate with males of Species A.
6. *Different population sizes.* There needs to be a ready supply of males of the other species; interspecific mating would be favoured in the less common or more patchily distributed species.
7. *Different intensities of intraspecific competition.* This will be associated with different mature colony sizes, colony lifespan and nest site turnover rate, and reproductive output.

A wide variety of types of social parasitism are found in the eusocial Hymenoptera, particularly in the ants (dulosis, temporary social parasitism,inquilinism, cleptobiosis, lestobiosis, parabiosis; see Hölldobler and Wilson, 1990). It would not be surprising, then, that if it was possible for "sperm parasitism" to be advantageous theoretically, that it would be found in at least

one ant species. Two reported cases of hybridization in ants are particularly worthy of consideration in the context of the model.

Pearson (1982, 1983a,b, 1987) has studied colonies with workers morphologically intermediate between Lasius niger and L. alienus in a heathland study area in England. Electrophoretic evidence indicated that the intermediate workers were F₁ hybrids resulting from the matings of L. alienus queens with L. niger males. In colonies with hybrid workers, the males produced appeared to be L. alienus as judged by morphological and electrophoretic evidence, indicating parthenogenetic production of males by L. alienus queens. There was no evidence of colony founding by F₁ hybrid queens. Intermediate nests were found in the area dominated by L. alienus colonies, but primarily at the edge of this area.

Pearson (1983b) suggested that the costs of producing infertile gynes by interspecifically mated queens could be partially compensated by the greater size of the hybrid workers compared to normal L. alienus workers, which might give them an advantage in establishing nests or foraging. In contrast, the hybrid workers were smaller than those of L. niger, and thus there might be no advantage to producing hybrid workers for a queen of L. niger mating with male L. alienus. Furthermore, while most or all of the males were produced by the queen in a colony with an L. alienus queen, in L. niger colonies workers lay some or all of the male eggs. Pearson (1983b) also noted that some colonies with L. alienus queens (both intraspecifically and interspecifically mated) produced males only for at least one year, and suggested that the greater size of hybrid workers may lead to increased male production.

Wing (1968) revised Acanthomyops, a genus endemic to North America which likely arises within the Lasius lineage (and hence leaves Lasius as a paraphyletic group by its recognition as a separate genus). Using morphological evidence, Wing recognized hybrids between five pairs of species. By far the most common hybrids found were A. latipes × claviger.

Wheeler and McClendon (1903) had interpreted the hybrid queens of this crossing as being the "alpha-form" queen of A. latipes, with the regular A. latipes queen being called the "beta-form" queen. Wing (1968) found that males produced in hybrid colonies were typical of those produced by A. latipes colonies, and thus concluded the hybridization was between an A. latipes queen and an A. claviger male. No evidence of backcrossing of the hybrids to either parent form was found, although Wing believed that such backcrossing would have been evident if it existed. Wing presented a plausible explanation for why hybrid queens might be unable to found colonies, even if they were fertile. The queens of A. latipes, like those of other species of Acanthomyops, are believed to found their colony by temporary social parasitism on Lasius colonies. The entry of an A. latipes queen into a Lasius colony is likely a highly risky procedure which involves complex behavioural responses to the actions of the Lasius workers. There is no reason to believe that a queen of A. latipes that mated interspecifically would act differently than a queen that mated intraspecifically, but an F₁ hybrid queen might very well exhibit intermediate behavioural responses which decrease the chances of successful adoption by the host colony.

Wing noted that records of the hybrid form were from a band from Minnesota and central Illinois east to New England and New Jersey, with records for both A. latipes and A. latipes × claviger absent from eastern Michigan to eastern New York. The pattern of widely scattered occurrences of the hybrids within a broad area of sympatry of the parent species does not fit the usual definitions of a "hybrid zone" (e.g., Barton and Hewitt, 1985; Bigelow, 1965; Hewitt, 1989) although Harrison and Rand's (1989) definition of a "mosaic hybrid zone" cannot be ruled out. It is somewhat difficult to determine from Wing's data what the relative abundance of the hybrid form is to the parent forms. Of the samples he examined, 25 contained only A. latipes × claviger queens, and one contained both A. latipes and A. latipes × claviger queens. In contrast, he identified 486 samples as A. claviger and 258 samples as A. latipes, but some of the

worker-only samples identified as A. latipes were probably hybrids, and many of the records were from areas outside of the zone of sympatry.

Mary Talbot's extensive studies on the ant fauna of the Edwin S. George Reserve in southeastern Michigan provide a better estimate of the relative abundance of the A. latipes × claviger hybrid. Talbot (1973) reported finding 110 colonies of Acanthomyops in over 20 years of study at the reserve: 2 subglaber, 11 murphyi, 13 interjectus, 28 claviger, and 56 latipes. Of the 25 latipes colonies in which females were found, six colonies were producing "alpha-type" (= latipes × claviger) queens. This provides a point estimate of the proportion of A. latipes colonies with interspecifically mated queens of 0.24, with a 95% confidence bound (using the common normal approximation) of 0.17. Even the lower limit of the confidence interval (0.07) represents a substantial proportion of hybrid colonies. Of course, we cannot infer what proportion of new queens mated interspecifically, since it is not known what the relative probabilities were of successfully founding a colony. Talbot (1973) does indicate that the hybrid colonies can be quite long-lived: one "alpha-latipes" colony that she had observed continuously for 21 years (1952 – 1972) was still producing males and females at the time she wrote her paper.

Other aspects of the natural history of the two Acanthomyops species appear to fit the model. Mature colonies are large (Talbot, 1963) and capable of immense alate production; Talbot estimated that 63,600 reproductives flew in four flights from a single colony of the related species Acanthomyops interjectus, and alates were still in the nest. Talbot (1973) reported that A. claviger was the latest of all five species in developing their alates. She further noted that "The distinctive feature of the A. claviger flights was that they occurred at lower temperatures than those of other Acanthomyops species at the reserve. They did not start until the temperature fell below 70°F and they continued until 64°F was reached. Thus they began at about the temperature when most flights of other species were ending." That A. latipes and A. claviger

must occasionally have mating flights at the same time is obvious if they are going to hybridize. Such an event was recorded by Wheeler and McClendon (1903); the two species were flying at exactly the same time from two nests described as being no more than twenty feet apart.

Sperm parasitism may be more likely to arise in ants than in other eusocial Hymenoptera. In many species of bees and wasps workers are more likely to produce a greater proportion of the males (Bourke, 1988). Intraspecific competition may be less intense in bees and wasps. There is also a substantially larger foraging territory for flying Hymenoptera, and thus hybrid workers of bees or wasps may not be able to exploit marginal habitats the way hybrid ant workers could. I know of no hybridization phenomena in wasps or bees which might fit the sperm parasitism scenario, although I have spoken to specialists in these groups (James Carpenter, pers. comm.; George Eickwort, pers. comm.).

Special problems are likely to exist for systematists in recognizing cases in nature which fit the model I have proposed, if indeed any such cases exist. The necessary requirement that the hybridizing species be sufficiently genetically compatible so that viable hybrid workers are produced makes it likely that the hybridizing species are closely related. This implies that the species are likely to be morphologically very similar, and perhaps sibling species. Since most ant taxonomy is based on workers (because they are collected much more frequently than the reproductives), it may be difficult to recognize the hybridizing species as being genetically isolated if morphologically intermediate hybrid workers are fairly common. The presence of hybrid queens presents a similar problem if this caste is used. If the males of the two species are morphologically distinct (for example, in genitalia) but the female castes are poorly differentiated, workers and alate gynes from hybrid colonies with associated males may be allocated to the species of the nest queen, rather than recognized as hybrids.

I am currently developing a more mathematically elaborate model for the evolution of sperm parasitism. A question of considerable interest is how sex ratio compensation should operate if sperm parasitism is occurring. Taylor (1981; see also Nonacs, 1986a) showed that queenright colonies should only compensate part-way in producing extra gynes when there is all-male production by orphaned queenless colonies in the population. Peter Taylor kindly derived the result that there should be no sex-ratio compensation if a queen does not "know" how she has mated. This result is undergoing further investigation.

The model described here is, I believe, the first to suggest that natural selection can favour interspecific mating and hybridization under the strict species definition of Key (1981). Previous models which have suggested an adaptive purpose for interspecific hybridization have required a less strict species definition that would allow introgression (e.g., Lewontin and Birch, 1966; Potts and Reid, 1988, 1990) or hybridogenesis (e.g., Bullini and Nascetti, 1990). Of course, sperm parasitism could also evolve under the same conditions if a less strict species definition is used, but recognition of the adaptive purpose of hybridization would be confounded by the presence of introgression. My model is particular to the eusocial Hymenoptera, and thus is not a general explanation for interspecific hybridization in organisms. [However, a similar type of argument might provide a plausible evolutionary explanation of hybridization in species with paternal care, such as in some freshwater fish. This hypothesis is currently under development.] Nonetheless it suggests another unusual route that natural selection might follow that, should it be confirmed as occurring in nature, would result in the discovery of a new type of social parasitism in the eusocial Hymenoptera. It also offers rich opportunities for further research along both theoretical and experimental avenues.

3.16. Concluding Remarks

The results presented in this thesis constitute, I believe, a significant advance in our understanding of the fulva-rudis-texana complex of Aphaenogaster. It should be emphasized that the species (or forms) recognized in this thesis were delineated by genetic markers that exhibit very little variability. The degree of morphological differentiation among forms was then examined, primarily using morphometric methods; morphology (including morphometrics) was not used to initially define the forms. Nonetheless the six forms recognized by Creighton (1950) are all recognized here as constituting valid species, although delimited somewhat differently; the problems involved with trying to identify the species using morphological characters have also been further clarified. Furthermore I have no evidence that Creighton synonymized any valid names (although the synonymy of silvestrii under texana is almost certainly incorrect). I believe that it might have been possible to recognize N16 and N22b on the basis of morphology and field behaviour, but Crozier (1975) was the first to recognize N16, which he did via karyology. The recognition of N17 and N19 probably would have been impossible strictly using worker morphology, although the structure of the male genitalia for providing taxonomic characters has not been properly investigated. Each of these forms is very close morphologically and karyotypically to another form (picea and carolinensis, respectively), so the status of these forms, which I am provisionally treating as distinct species, requires further testing. The morphometric analysis on the genetically identified colonies has provided a basis for judging the taxonomic status of the names punctaticeps (as a valid species) and crinimera (as a synonym of texana), although no live colonies attributable to these names were seen in this study.

Phenetically, fulva is sufficiently distinct morphologically that it seems reasonable to refer to the set of species consisting of the fulva-rudis-texana complex with fulva removed as the rudis-texana subcomplex. This is supported by chromosomal and electrophoretic evidence. The

karyotype of fulva cannot be easily derived from any other karyotype studied, and fulva is certainly distinct in PGM-1,2. However, the finding of Crozier (1977) suggesting limited gene flow between fulva and N22a (rudis) in Georgia would indicate that fulva is not greatly different from the rudis-texana subcomplex members. If Crozier's evidence of limited gene flow between could be verified, it would suggest that in view of the broad sympatry between fulva and other fulva-rudis-texana complex members (particularly rudis), the species definition of Key may be too rigid as a basis for practical taxonomic decisions.

Although several characters can be used to separate fulva from the rudis-texana subcomplex, none of them seem to provide any cladistic information indicating fulva's phylogenetic position with respect to the other species. An evolutionary biologist (*sensu* Mayr, 1963) would place fulva outside of the rudis-texana subcomplex, and I believe this is most likely its correct phylogenetic placement. However, no character clearly indicates that the rudis-texana subcomplex is monophyletic.

Further research within the complex requires expanding the sampling geographically, particularly through the midwest, and more intensive sampling within the range covered, particularly within Florida. Sample sizes of several forms should be expanded in order to increase the robustness of the discriminant functions; the unusual heterogeneity of miamiana (N21a) in particular requires further sampling and study. Intensive genetic studies at sites of sympatry (of which several were identified in this study), designed to detect gene flow, will be required to further test for reproductive isolation between pairs of putative species.

It would be best to continue this research with the goal of revising Aphaenogaster on a world-wide basis. A reasonable intermediate objective would be to complete a systematic revision of the New World Aphaenogaster; at this point museum collections could be used profitably, but the option to use the methodologies employed in my thesis research should be

available. Among species outside of the fulva-rudis-texana complex, karyotyping should be highly informative on the status of the forms treated as valid subspecies by Smith (1979). These include smithi, treated as a subspecies of boulderensis, and pluteicornis, treated as a subspecies of treatae. Particularly interesting will be an examination of the relationship of occidentalis and valida to one another, and to the form which Creighton (1950) treated them as subspecies of, the European subterranea. Roy Snelling (pers. comm.) believed that valida is a junior synonym of occidentalis, which is in turn specifically distinct from subterranea, but Creighton (1950, p. 149) believed that "it seems virtually certain that the name occidentalis will have to go into the synonymy of subterranea" when enough material of the latter was examined. In section 1.4 it was noted that Hauschteck (1962) has already obtained an $n = 11$ karyotype for subterranea from Switzerland. W. L. Brown's hypothesis (pers. comm.) that the Central American Aphaenogaster with ergatoid queens are conspecific (with araneoides falling as a synonym of phalangium) will be another interesting test of this methodology.

A great advantage of the genetic approach is that it avoids the circularity of using morphology to both delineate and classify several closely related species and hence avoids inevitable misclassifications which affect one's concepts of the species. In most taxonomic studies, the morphological gaps between species are sufficiently great that this circularity does not arise. In these cases, so-called "alpha-taxonomic" methods will be sufficient, and indeed it should be argued that they are the most rapid and cost-effective ways of meeting the urgent need to catalogue our dwindling biodiversity. However, there are clearly situations where morphology cannot resolve a problem satisfactorily, and other methods must be called upon. These methods will inevitably be slower or more expensive, but the resolution of sibling species complexes is of great theoretical and often practical importance.

In my thesis research, the karyotype data is more extensive and proved far better at delineating the component forms than did electrophoresis. This is not an entirely fair assessment of electrophoresis as a tool for sorting out sibling species complexes, even in the fulva-rudis-texana complex. The bulk of my research time was spent on karyology, since it was producing clear results at a rapid pace, and it was highly demanding since karyotypes had to be prepared when brood became available. Unlike electrophoresis, where material could be frozen for analysis at a more convenient time, the karyological technique required live material. Most of the northern forms refused to rear brood through to a stage optimal for preparing karyotypes much after mid-August without undergoing a diapause period; this is shortly after the alates are produced, and hence there is a rather narrow "window" for handling colonies for optimal data collection.

The electrophoretic work was conducted after most of the karyotypic and morphometric work was already completed, hence very specific questions could be addressed. Nonetheless I was also quite interested in obtaining further genetic characters for delineating each form, since preparing karyotypes is a rather laborious procedure for verifying the identity of multiple colonies. I was also interested in the identification of cladistically useful characters for phylogenetic analysis. Although the realization of these other goals is somewhat limited to date, I believe that much more information is available from electrophoresis, particularly using alternative methodologies. However, I also believe that DNA methodologies or further chromosomal analysis using banding protocols are likely to provide better indicators of phylogenetic affinity.

I have presented here a model suggesting that interspecific mating in ants (or other eusocial Hymenoptera) with the subsequent production of infertile hybrid offspring can be selected for as part of a mixed evolutionarily stable strategy. I believe this is the first such model

in biology which can give an adaptive function to interspecific hybridization under a strict species definition that allows no introgression. The use of the term "sperm parasitism" should prove to be controversial, but I believe it is an apt descriptor of the phenomenon, as it would constitute a new mode of social parasitism between ant species. Although the existence of this phenomenon in nature has yet to be proven, the examples discussed are quite suggestive. At the very least, the model offered will give ant taxonomists trying to resolve difficult species complexes another theoretical possibility to consider.

Although the systematic analysis of any group of organisms is a worthwhile endeavour, it could be argued that the sheer abundance of members of the fulva-rudis-texana complex makes their taxonomic resolution particularly desirable. Moreover, this abundance, combined with the relative ease of rearing them in the laboratory, suggests that this complex may be an excellent haplodiploid model for basic research in cytogenetics, population genetics, niche partitioning, processes of speciation, and morphometrics. A modest investment of resources into further research on these interesting ants should continue to pay intellectual dividends.

Table 1. Names in, or associated with, the fulva-rudis-texana complex of the ant genus Aphaenogaster, listed in order of first description. Citation for original description, information on types, and miscellaneous notes given. Abbreviations: M = male(s), W = worker(s), Q = queen(s).

opposita

Myrmica opposita Say, 1836: 292.

Type locality: North America. Indiana?

Type material: unknown number of M, W.

Location of types: probably lost.

Notes: Smith (1979) listed this as an unplaced taxon of the Myrmicinae. Emery (1921) considered it to be a good species of Aphaenogaster. It was not mentioned by Creighton (1950).

fulva

Aphaenogaster fulva Roger, 1863: 190.

Type locality: North America.

Type material: unknown number of W.

Location of types: probably in Roger collection, Museum für Naturkunde an der Humboldt-Universität zu Berlin, Zoologisches Museum.

Notes: recognized as a valid species by Creighton (1950).

aquia

Myrmica (Monomarium (!)) aquia Buckley, 1867: 341.

Type locality: Aquia, Virginia, and western New York.

Type material: unknown number of W, Q.

Location of types: none known to exist.

Notes: Creighton (1950) considered this to be an unrecognizable form.

rudis

Stenamma (Aphaenogaster) fulvum aquia var. rudis Emery, 1895: 305.

Type locality: Virginia, by restriction of Creighton (1950). Original description also included the District of Columbia and North Carolina.

Type material: unknown number of W, Q.

Location of types: assumed to be in the Emery collection, Museo Civico di Storia Naturale 'Giacomo Doria', Genoa, Italy.

Notes: Creighton considered this to be a valid species.

azteca

Stenamma (Aphaenogaster) fulvum aquia var. aztecum Emery, 1895: 305.

Type locality: Mexico.

Type material: unknown number of W.

Location of types: same as rudis.

Table 1 (cont'd)

picea

Stenamma (Aphaenogaster) fulvum aquia var. piceum Emery, 1895: 305.

Type locality: Connecticut, by restriction of Creighton (1950). Original description also included Canada, Pennsylvania, District of Columbia, New York, and New Jersey.

Type material: unknown number of W, M, Q.

Location of types: same as rudis.

Notes: Creighton (1950) considered this to be a northern and high elevation subspecies of rudis.

pusilla

Stenamma (Aphaenogaster) fulvum aquia var. pusillum Emery, 1895: 306.

Type locality: Washington, DC.

Type material: unknown number of W.

Location of types: same as rudis.

Notes: Creighton (1950) considered this to be an unrecognizable form, as it was described from minors only.

texana

Stenamma (Aphaenogaster) fulvum aquia var. texana Emery, 1895: 306.

Type locality: Texas.

Type material: 2 W.

Location of types: same as rudis.

Notes: Creighton (1950) considered this to be a good species.

furvescens

Aphaenogaster texana var. furvescens Wheeler, 1915: 413.

Type locality: Miller Canyon, 5800', and Ramsay Canyon, 5800', Huachuca Mountains, Arizona.

Type material: "several" W and one dealate Q.

Location of types: MCZ, AMNH.

Notes: Creighton (1950) synonymized this form with texana.

carolinensis

Aphaenogaster texana var. carolinensis Wheeler, 1915: 414.

Type locality: Tryon, North Carolina.

Type material: described from one dealate Q and "numerous workers taken from several small colonies nesting under stones in open woods".

Location of types: MCZ, AMNH.

Notes: Creighton (1950) treated carolinensis as a subspecies of texana. Carroll (1975) treated carolinensis as a separate species, to include all rudis-texana forms (including miamiana) from Florida.

Table 1 (cont'd)

flemingi

Aphaenogaster texana flemingi M. R. Smith, 1928: 275.

Type locality: A & M College, Mississippi.

Type material: "many" W (from nest of 90 W?)

Location of types: USNM, Dept. of Entomology at A & M College, MCZ.

Notes: Creighton (1950) treated this as a valid species.

silvestrii

Aphaenogaster (Deromyrma) Silvestrii Menozzi, 1929: 282, fig. 1.

Type locality: Gainesville, Florida.

Type material: one W, one Q.

Location of types: probably in the Istituto di Entomologia dell'Università, Bologna, Italy.

Notes: Creighton (1950) synonymized this form with texana.

miamiana

Aphaenogaster (Attomyrma) texana var. miamiana Wheeler 1932: 5.

Type locality: Miami, Florida. Other specimens from Paradise Key, Planter on Key Largo, Biscayne Bay.

Type material: 8 W, 3 Q, 1 M from Miami, plus other specimens of dubious status.

Location of types: MCZ, AMNH.

Notes: Creighton (1950) treated miamiana as a good species. Carroll (1975) treated miamiana as a synonym of carolinensis.

nana

Aphaenogaster (Attomyrma) texana nana Wheeler, 1932: 6.

Type locality: Gainesville, Florida.

Type material: 3 W.

Location of types: MCZ, AMNH.

Notes: Creighton (1950) considered this to be an unrecognizable form, as it was described from minims only.

macrospina

Aphaenogaster texana macrospina M. R. Smith, 1934: 386, fig. 1, 2.

Type locality: The Navy Yard, Charleston, South Carolina.

Type material: 4 W.

Location of types: USNM.

Notes: Creighton (1950) treated macrospina as a valid species, but it was synonymized with flemingi by Smith (1957).

Table 1 (cont'd)

huachucana

Aphaenogaster (Attomyrma) huachucana Creighton, 1934: 189.

Type locality: Ramsey Canyon, Huachuca Mountains, Arizona, 7000'.

Type material: 90 W.

Location of types: LACM, MCZ, AMNH, USNM.

Notes: Creighton (1934) considered this species to be closely related to texana. Creighton (1950) treated this as a valid species.

punctithorax

Aphaenogaster texana punctithorax Cole, 1938: 239.

Type locality: Gregory's Bald, 3500', Great Smoky Mountains National Park, Tennessee.

Type material: holotype W and 4 paratype W from a single nest.

Location of types: presently missing; may be in alcohol at LACM.

Notes: Creighton (1950) considered this to be an unrecognizable form, as it was described from minors only.

rubida

Aphaenogaster fulva var. rubida Enzmann, 1947: 147, fig.

Type locality: Arnold Arboretum, Boston, Massachusetts.

Type material: unknown number of W; one is designated "Holotype No. 12".

Location of types: Enzmann collection, whereabouts unknown.

Notes: Creighton (1950) synonymized this form with fulva.

crinimera

Aphaenogaster (Allomyrma (!)) huachucana crinimera Cole, 1953: 82, fig. 1.

Type locality: campground area at Bandelier National Monument, New Mexico, elev. 6050'.

Type material: holotype W and a large series of paratype W, M, Q from colony J-185.

Location of types: LACM (probably has holotype), USNM, MCZ, AMNH, several private collections.

punctaticeps

Aphaenogaster punctaticeps MacKay, 1989: 47, fig. 1-7.

Type locality: Jornada Experimental Range, Dona Ana Co., New Mexico.

Type material: holotype W and one paratype W.

Location of types: USNM (holotype), LACM (paratype).

Table 2. Localities from which one or more *Aphaenogaster* colonies were karyotyped, and corresponding locality code. Colonies were collected by the author unless otherwise specified.

CODE	LOCALITY
ON1	CANADA. Ontario: Chaffey's Locks
ON2	Ont.: Constance Bay (30 km W Ottawa)
ON3	Ont.: 15 km E Gananoque
ON4	Ont.: 6 km W Halls Lake, near Lake Kushog
ON5	Ont.: Pinery Provincial Park (4 km S Grand Bend)
ON6	Ont.: Rondeau Provincial Park (15 km S Ridgeway)
AL1	USA. Alabama: Cleburne Co., Cheaha State Park, 2300'
AZ1	Arizona: Cochise Co., 13 km WNW Portal, Pinery Canyon, Chiricahua Mts, 6200', coll. S. Cover
AZ2	AZ: Pima Co., 16 km NE Tucson, Bear Canyon, Santa Catalina Mts, 6000', coll. S. Cover
CT1	Connecticut: Hartford Co., West Suffield, coll. L. Ling
FL1	Florida: Alachua Co., 8 km E Gainesville (E shore of Newnan's Lake)
FL2	FL: Franklin Co., 7 km S Sopchoppy (E bank of Ochlocknee River)
FL3	FL: Highlands Co., Archbold Biological Station
FL4	FL: Lake Co., Ocala National Forest, 8 km N Altoona
FL5	FL: Walton Co., 7.5 km W Bruce
FL6	FL: Walton Co., 6 km E Bruce, W bank of Choctawatchee River
GA1	Georgia: Greene Co., Oconee National Forest, 6 km N Greensboro
GA2	GA: Montgomery Co., 14 km N Mount Vernon
GA3	GA: Rabun Co., Black Rock Mountain State Park
GA4	GA: Toombs Co., Vidalia
IN1	Indiana: Bartholomew Co., 20 km SW Columbus
IN2	IN: Orange Co., Hoosier National Forest, 2 km SE Paoli, Pioneer Mothers Memorial Forest
IN3	IN: Perry Co., Hoosier National Forest, 3.5 km SE Oriole
KY1	Kentucky: Laurel Co., Daniel Boone National Forest, Bald Rock Recreation Area
KY2	KY: Rowan Co., Morehead, Daniel Boone National Forest, Rodburn Hollow Recreation Area
MD1	Maryland: Calvert Co., 8 km S Prince Frederick
MD2	MD: Washington Co., near Hagerstown
MO1	Missouri: Callaway Co., Graham Cave State Park, coll. J. Trager
MO2	MO: St. Louis Co., Eureka, coll. J. Trager

Table 2 (cont'd).

NC1	North Carolina: Davie Co., 4 km N Mocksville
NC2	NC: Forsyth Co., Rural Hall
NC3	NC: Haywood Co., Pisgah National Forest, 8 km SE Waterville
NC4	NC: Iredell Co., Duke Power State Park
NC5	NC: Montgomery Co., Uwharrie National Forest, 8 km SW Troy
NC6	NC: Moore Co., 3 km N Southern Pines
NC7	NC: Moore Co., Whispering Pines
NC8	NC: Stanly Co., Morrow Mountain State Park
NC9	NC: Swain Co., 8 km E Bryson City
NC10	NC: Swain Co., Great Smoky Mountains National Park, Newfound Gap, 5048', coll. J. M. Cumming
NC11	NC: Wilkes Co., Stone Mountain State Park
NJ1	New Jersey: Burlington Co., Bass River State Forest
NJ2	NJ: Burlington Co., Wharton State Forest, 1 km E Atsion
NJ3	NJ: Mercer Co., Princeton
NJ4	NJ: Middlesex Co., New Brunswick
NJ5	NJ: Warren Co., Worthington Forest
NY1	New York: Saratoga Co., Saratoga Spa State Park
OH1	Ohio: Lawrence Co., Wayne National Forest, 4 km E Pedro, Lake Vesuvius Recreation Area, Sand Hill
OH2	OH: Morgan Co., Wayne National Forest, 20 km W Stockport, Wildcat Hollow
PA1	Pennsylvania: Butler Co., Moraine State Park
PA2	PA: Cumberland Co., Dickinson
PA3	PA: Perry Co., 8.7 km S Millersburg, Notch Road, 4.5 km W Hwk 11-15
PA4	PA: Schuylkill Co., 4 km W Pine Grove
PA5	PA: Sullivan Co., Wyoming State Forest
SC1	South Carolina: Beaufort Co., Hunting Island State Park
SC2	SC: Berkeley Co., Francis Marion National Forest
SC3	SC: Chesterfield Co., Carolina Sandhills National Wildlife Refuge
SC4	SC: Colleton Co., Colleton State Park
TN1	Tennessee: Cumberland Co., 5 km W Crab Orchard
TN2	TN: Knox Co., Knoxville
TX1	Texas: Travis Co., vicinity of Austin [The single colony from here had been reared at the University of Texas for at least three years, and was kindly sent to me by Sandford Porter.]
VA1	Virginia: Roanoke Co., Roanoke
WV1	West Virginia: Braxton Co., Servia
WV2	WV: Preston Co., 13 km W Bruceton Mills, Coopers Rock State Forest

Table 3. Analysis of short arms for representative karyotypes. All chromosomes classified as acrocentrics or pseudoacrocentrics, plus selected small metacentrics, included. Adjustment factors use tennesseensis karyotype as a standard.

form	No. of short arms	sum of R_s values	mean R_s value	adjustment factor	excluded chromosomes
N16	9	9.57	1.06	1.04	1-2, 5-7
N17	14	16.58	1.18	1.12	1-2, 4-5
N18	15	15.99	1.07	1.12	1, 3, 5
<u>fulva</u>	14	21.29	1.52	1.19	1, 3-4, 6
N19	17	17.73	1.04	1.14	1-2
N20	18	20.72	1.15	1.26	1-2
N21a	20	22.64	1.13	1.21	1
N21b	21	25.22	1.20	1.25	none
N22a	22	26.83	1.22	1.28	none
N22b	22	22.64	1.03	1.21	none
<u>lamellidens</u>	16	25.75	1.61	1.26	1, 3-4, 6
<u>tennesseensis</u>	5	6.30	1.26	1.00	1-8
<u>treatae</u>	20	24.45	1.23	1.24	2

Table 4. Enzymes screened at CLEEG (Cornell Laboratory for Ecological and Evolutionary Genetics) on four buffer systems (gels). Although SOD was not stained for specifically, it came up on some other stains. The gels on which promising activity was observed is listed; brackets indicate weaker activity or poorer resolution on that gel. Enzyme systems with no gel listed were considered to have limited activity or very poor resolution on all gels. The same enzyme was stained for by the FBP and G6PDH stains. Table adapted from May *et al.* (1988); this source also contains all recipes used.

Abbrev.	Enzyme name	Gel
ACP	Acid phosphatase	R
ADA	Adenosine deaminase	
AK	Adenylate kinase	
ADH	Alcohol dehydrogenase	
ALD	Aldolase	C
AKP	Alkaline phosphatase	
AAT	Aspartate aminotransferase	C
CAT	Catalase	
DIA	Diaphorase	R
EST AS-D	Esterases	R
EST-F-A	Esterases	
FBP	Fructose biphosphatase	R
FUM	Fumarase	
β -GAL	β -galactosidase	
GAM	Galactosaminidase	R
GK	Glucokinase	R, (4)
G6PDH	Glucose-6-phosphate dehydrogenase	R
GPI	Glucosephosphate isomerase	R, (4)
α -GLU	α -glucosidase	C
β -GLU	β -glucosidase	
GDH	Glutamic dehydrogenase	R
GR	Glutathione reductase	R
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	C
G3P	Glycerol-3-phosphate dehydrogenase	

Table 4 (cont'd).

Abbrev.	Enzyme name	Gel
GDA	Guanine deaminase	
HA	Hexoseaminidase	R
HBDH	Hydroxybutyric dehydrogenase	S 9
IDH	Isocitrate dehydrogenase	4
LDH	Lactate dehydrogenase	R
LAP	Leucine aminopeptidase	R
MDH	Malate dehydrogenase	4, (S-9)
ME	Malic enzyme	C, (4)
α -MAN	α -mannosidase	R
MPI	Mannosephosphate isomerase	
MUP	Methylumbelliferyl phosphatase	
NP	Nucleoside phosphorylase	
ODH	Octanol dehydrogenase	
PEP-GL	Peptidase with glycyl-leucine	R
PEP-LA	Peptidase with leucyl-alanine	
PEP-LGG	Peptidase with leucyl-glycyl-glycine	
PEP-PAP	Peptidase with phenyl-alanyl-proline	R
PGM	Phosphoglucomutase	R, 4, S-9
PGD	Phosphogluconate dehydrogenase	4
PGK	Phosphoglycerate kinase	C
PRO	General protein	
PP	Inorganic pyrophosphatase	S-9
SOD	Superoxide dismutase	R
TPI	Triosephosphate isomerase	
XDH	Xanthine dehydrogenase	R

Table 5. Summary univariate statistics for each form. Var. = variable, Stat. = statistic, N = sample size, mean = sample mean, min = minimum observation in sample, max = maximum observation in sample, CV = coefficient of variation (%). All measurements are in mm.

Var.	Stat.	fulva	N16	N17	N18	N19	N20	N21a	N21b	N22a	N22b
HW	N	38	15	33	39	5	32	13	13	67	9
	mean	0.99	1.00	0.95	0.97	0.82	0.87	0.94	0.91	0.90	1.03
	min	0.81	0.80	0.82	0.80	0.71	0.64	0.85	0.75	0.70	0.84
	max	1.16	1.12	1.15	1.11	0.97	1.05	1.03	1.06	1.07	1.18
HL	CV	8.31	8.84	7.58	7.57	12.55	11.53	5.81	10.68	9.02	11.86
	mean	1.20	1.21	1.17	1.18	1.12	1.15	1.22	1.27	1.16	1.28
	min	1.04	1.02	1.06	1.03	1.03	0.86	1.12	1.08	0.97	1.10
	max	1.36	1.34	1.36	1.32	1.24	1.32	1.32	1.44	1.32	1.41
SL	CV	6.13	7.01	6.02	5.82	7.25	8.61	4.42	8.76	6.05	8.38
	mean	1.30	1.28	1.35	1.34	1.34	1.32	1.41	1.49	1.32	1.38
	min	1.12	1.14	1.23	1.20	1.26	1.06	1.32	1.31	1.16	1.21
	max	1.46	1.38	1.51	1.44	1.47	1.46	1.52	1.64	1.46	1.48
EL	CV	5.71	5.33	4.24	4.02	6.27	6.72	4.67	6.82	5.07	6.99
	mean	0.24	0.25	0.24	0.24	0.24	0.24	0.28	0.24	0.24	0.25
	min	0.20	0.20	0.21	0.20	0.20	0.18	0.24	0.20	0.18	0.22
	max	0.28	0.27	0.30	0.27	0.28	0.30	0.30	0.28	0.27	0.28
WLA	CV	7.52	8.25	7.63	7.52	12.57	10.30	5.72	9.26	7.89	8.81
	mean	1.61	1.59	1.61	1.62	1.53	1.55	1.65	1.71	1.55	1.70
	min	1.40	1.34	1.42	1.42	1.42	1.20	1.52	1.46	1.28	1.46
	max	1.78	1.72	1.88	1.80	1.72	1.76	1.76	1.92	1.74	1.84
MH	CV	6.02	6.61	6.18	5.71	7.89	8.29	4.02	8.24	6.10	8.38
	mean	1.01	1.03	1.02	1.05	0.96	0.98	1.05	1.09	0.99	1.10
	min	0.84	0.84	0.86	0.86	0.86	0.70	0.96	0.88	0.76	0.92
	max	1.20	1.12	1.24	1.20	1.10	1.14	1.14	1.24	1.14	1.22
	CV	7.55	7.92	7.67	7.29	9.97	9.75	4.98	11.24	7.56	10.40

Table 5 (cont'd).

Var.	Stat.	fulva	N16	N17	N18	N19	N20	N21a	N21b	N22a	N22b
	N	38	15	33	39	5	32	13	13	67	9
SPL	mean	0.34	0.27	0.31	0.31	0.26	0.27	0.34	0.25	0.26	0.29
	min	0.22	0.19	0.27	0.24	0.22	0.18	0.28	0.20	0.19	0.22
	max	0.42	0.33	0.40	0.40	0.31	0.33	0.40	0.30	0.32	0.36
	CV	12.38	12.09	10.20	9.48	13.70	13.14	11.31	11.45	11.89	15.67
ISPL	mean	0.21	0.14	0.17	0.17	0.12	0.14	0.20	0.09	0.14	0.15
	min	0.11	0.09	0.14	0.13	0.09	0.06	0.16	0.05	0.07	0.10
	max	0.28	0.18	0.22	0.24	0.16	0.18	0.25	0.12	0.19	0.20
	CV	17.14	16.20	12.72	11.94	22.82	18.86	13.10	21.57	18.67	23.03
SPD	mean	0.34	0.30	0.31	0.32	0.26	0.28	0.33	0.30	0.28	0.28
	min	0.26	0.22	0.24	0.25	0.22	0.20	0.30	0.25	0.22	0.23
	max	0.43	0.35	0.40	0.39	0.30	0.33	0.36	0.38	0.36	0.32
	CV	13.19	9.57	12.60	10.76	14.39	11.15	6.05	13.32	9.94	11.06
FL	mean	1.67	1.56	1.60	1.60	1.59	1.59	1.71	1.85	1.57	1.65
	min	1.43	1.30	1.41	1.39	1.44	1.24	1.54	1.55	1.30	1.44
	max	1.90	1.68	1.83	1.77	1.76	1.79	1.88	2.10	1.74	1.76
	CV	6.14	6.38	5.83	5.40	7.56	8.19	5.93	8.99	5.76	7.37
FW	mean	0.23	0.26	0.25	0.25	0.24	0.24	0.25	0.27	0.25	0.27
	min	0.20	0.22	0.22	0.22	0.22	0.18	0.23	0.22	0.21	0.24
	max	0.26	0.29	0.28	0.28	0.26	0.28	0.27	0.32	0.28	0.29
	CV	6.88	7.21	5.96	6.07	6.43	8.79	5.59	11.12	6.30	6.14
TL	mean	1.15	1.06	1.08	1.08	1.10	1.08	1.18	1.29	1.07	1.14
	min	0.98	0.86	0.95	0.94	0.99	0.84	1.06	1.06	0.87	0.96
	max	1.28	1.14	1.26	1.21	1.24	1.22	1.32	1.48	1.18	1.20
	CV	6.36	6.82	6.04	5.19	8.61	8.29	6.09	9.64	5.75	7.75

3 1 of/de 3

PM-1 3 1/2"x4" PHOTOGRAPHIC MICROCOPY TARGET
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Table 11. Classification results (using jackknife) for linear discriminant analysis, regular data set log transformed.

From	Number of individuals (percentage in italics) classified into form:									
	<i>fulva</i>	N16	N17	N18	N19	N20	N21a	N21b	N22a	N22b
<i>fulva</i>	38 <i>100.0</i>	0	0	0	0	0	0	0	0	0
N16	0	13 <i>86.7</i>	0	0	0	0	0	0	1 <i>6.7</i>	1 <i>6.7</i>
N17	0	0	27 <i>81.8</i>	6 <i>18.2</i>	0	0	0	0	0	0
N18	0	0	14 <i>35.9</i>	24 <i>61.5</i>	0	0	0	0	1 <i>2.6</i>	0
N19	0	0	0	0	4 <i>80.0</i>	1 <i>20.0</i>	0	0	0	0
N20	0	0	0	0	6 <i>18.8</i>	22 <i>68.8</i>	0	0	4 <i>12.5</i>	0
N21a	0	0	0	0	0	0	13 <i>100.0</i>	0	0	0
N21b	0	0	0	0	1 <i>7.7</i>	0	0	12 <i>92.3</i>	0	0
N22a	0	0	6 <i>9.0</i>	1 <i>1.5</i>	1 <i>1.5</i>	5 <i>7.5</i>	0	0	51 <i>76.1</i>	2 <i>3.0</i>
N22b	0	0	0	0	0	0	0	0	1 <i>11.1</i>	8 <i>88.9</i>

Table 12. Classification results (using jackknife) for linear discriminant analysis, regular data set log transformed, using reduced set of seven variables.

From	Number of individuals (percentage in italics) classified into form:									
	<i>fulva</i>	N16	N17	N18	N19	N20	N21a	N21b	N22a	N22b
<i>fulva</i>	38 <i>100.0</i>	0	0	0	0	0	0	0	0	0
N16	0	14 <i>93.3</i>	0	0	0	0	0	0	0	1 <i>6.7</i>
N17	0	0	23 <i>69.7</i>	9 <i>27.3</i>	0	0	0	0	1 <i>3.0</i>	0
N18	0	0	12 <i>30.8</i>	25 <i>64.1</i>	0	0	0	0	1 <i>2.6</i>	1 <i>2.6</i>
N19	0	0	0	0	3 <i>60.0</i>	1 <i>20.0</i>	1 <i>20.0</i>	0	0	0
N20	0	0	0	0	9 <i>28.1</i>	17 <i>53.1</i>	0	0	6 <i>18.8</i>	0
N21a	0	0	0	0	0	1 <i>7.7</i>	12 <i>92.3</i>	0	0	0
N21b	0	0	0	0	0	0	0	13 <i>100.0</i>	0	0
N22a	0	0	5 <i>7.5</i>	6 <i>9.0</i>	1 <i>1.5</i>	6 <i>9.0</i>	0	0	44 <i>65.7</i>	5 <i>7.5</i>
N22b	0	0	0	0	0	0	0	0	1 <i>11.1</i>	8 <i>88.9</i>

Table 13. Selected results of canonical variate analysis for the fulva-rudis-texana complex in Aphaenogaster. Log transformed regular data set with (a) all ten forms included and (b) fulva and N21b excluded. Note: % VAR = % of among-groups variation explained by the particular canonical variable.

	(a) All Ten Forms Included			(b) <u>fulva</u> and N21b Excluded		
	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
Raw Canonical Coefficients						
HW	-25.83	-14.84	-22.35	-30.60	13.74	10.92
HL	11.84	17.65	-19.31	5.09	-44.47	19.73
SL	18.76	-13.99	32.69	16.52	18.63	-30.99
EL	2.67	4.74	17.67	18.64	-7.19	14.84
MH	8.99	-13.41	4.62	-6.84	-0.49	-14.34
WLA	6.18	-1.25	-2.17	-1.65	11.04	-12.22
SPL	2.38	3.60	1.88	2.33	6.76	-2.00
ISPL	-6.98	-3.11	4.81	2.27	3.19	3.00
SPD	-3.76	3.23	1.48	1.70	4.66	4.08
FL	-5.28	19.07	-8.42	2.73	-5.62	-3.07
FW	16.41	-17.23	-0.26	-8.58	-4.18	-11.84
TL	-5.45	22.99	-8.81	6.64	-3.06	9.55
Standardized Canonical Coefficients						
HW	-2.69	-1.54	-2.32	-3.19	1.43	1.34
HL	0.86	1.29	-1.41	0.36	-3.16	1.40
SL	1.18	-0.88	2.06	0.93	1.05	-1.74
EL	0.24	0.42	1.58	1.71	-0.66	1.36
MH	0.78	-1.18	0.41	-0.59	-0.04	-1.24
WLA	0.44	-0.09	-0.15	-0.12	0.77	-0.86
SPL	0.38	0.58	0.30	0.34	1.00	-0.30
ISPL	-1.96	-0.88	1.35	0.51	0.71	0.67
SPD	-0.51	0.44	0.20	0.21	0.58	0.51
FL	-0.40	1.45	-0.64	0.18	-0.38	-0.21
FW	1.34	-1.41	-0.02	-0.61	-0.30	-0.85
TL	-0.44	1.87	-0.72	0.46	-0.21	0.66
% VAR:	51.2	23.9	13.8	49.6	34.4	8.5
Class Means of Canonical Variables						
<u>fulva</u>	-5.64	2.77	-1.11			
N16	-0.43	-2.34	-3.04	-3.45	-1.61	1.98
N17	-0.47	-1.73	1.07	-0.27	2.05	-0.38
N18	-1.02	-1.75	0.17	-1.12	2.11	0.00
N19	3.35	2.21	1.96	3.30	-1.59	-1.81
N20	1.52	0.94	1.32	1.98	-1.18	-0.02
N21a	-0.29	2.40	4.19	4.95	0.92	1.79
N21b	8.23	4.94	-2.49			
N22a	1.35	-0.93	-0.11	-0.28	-1.07	-0.42
N22b	1.92	-2.12	-2.25	-2.37	-2.30	-0.33

Table 14. Results of canonical variate analysis on three subsets of forms from the *fulva-rudis-texana* complex in *Aphaenogaster*. The three forms in each subset are: (a) N16, N22a, N22b; (b) N17, N18, N22a; (c) N20, N21a, N22b. Notes: data log transformed; % VAR = percentage of among-groups variation explained by the particular canonical variable.

	(a) N16, N22a, N22b		(b) N17, N18, N22a		(c) N20, N21a, N22a	
	CAN1	CAN2	CAN1	CAN2	CAN1	CAN2
Raw Canonical Coefficients						
HW	-24.69	22.31	22.56	-16.84	-15.17	18.08
HL	-9.44	-29.43	-40.47	-4.27	-5.85	-10.83
SL	31.77	-11.57	13.18	29.33	3.06	17.73
EL	-2.57	8.30	-7.22	13.61	21.17	-6.55
MH	2.84	-7.83	3.71	-15.15	-8.73	-10.17
WLA	-27.74	-27.95	11.04	-7.97	1.49	-8.51
SPL	3.01	-4.43	10.48	10.67	6.14	-8.32
ISPL	0.43	2.97	-0.65	-0.41	1.21	8.62
SPD	3.27	9.49	3.16	-4.09	5.11	5.75
FL	36.33	18.21	-7.38	10.54	1.50	-27.38
FW	-5.74	-3.80	-5.79	8.85	-16.80	4.82
TL	7.42	10.46	-12.08	-13.16	7.49	12.09
Standardized Canonical Coefficients						
HW	-2.65	2.39	2.03	-1.51	-1.52	1.82
HL	-0.68	-2.13	-2.45	-0.26	-0.42	-0.78
SL	1.79	-0.65	0.62	1.38	0.19	1.07
EL	-0.22	0.71	-0.57	1.07	2.12	-0.66
MH	0.25	-0.68	0.30	-1.21	-0.75	-0.87
WLA	-1.95	-1.96	0.70	-0.51	0.10	-0.60
SPL	0.39	-0.58	1.49	1.51	0.92	-1.24
ISPL	0.09	0.60	-0.13	-0.08	0.29	2.06
SPD	0.34	0.98	0.39	-0.50	0.60	0.67
FL	2.29	0.77	-0.43	0.61	0.11	-1.98
FW	-0.40	-0.27	-0.36	0.55	-1.23	0.35
TL	0.48	0.67	-0.69	-0.76	0.56	0.91
% VAR:	83.0	17.0	90.2	9.8	91.3	8.7
Class Means of Canonical Variables						
N16	-3.77	0.94				
N17			1.39	0.85		
N18			1.73	-0.65		
N20					0.87	-0.90
N21a					4.62	0.82
N22a	1.11	0.12	-1.69	-0.04	-1.31	0.27
N22b	-2.01	-2.45				

Table 15. Square roots of Mahalanobis' generalized distances (D^2) between forms of the fulva-rudis-texana complex in Aphaenogaster. Regular data set, log transformed.

	<u>fulva</u>	N16	N17	N18	N19	N20	N21a	N21b	N22a	N22b
<u>fulva</u>	0	7.86	7.35	6.81	9.59	7.88	7.74	14.25	8.02	9.40
N16	7.86	0	5.39	4.88	8.16	5.99	8.72	11.98	4.17	3.64
N17	7.35	5.39	0	1.50	5.95	4.26	5.92	11.55	3.28	5.31
N18	6.81	4.88	1.50	0	6.53	4.76	6.57	11.74	3.57	5.27
N19	9.59	8.16	5.95	6.53	0	2.74	4.93	7.61	4.52	6.76
N20	7.88	5.99	4.26	4.76	2.74	0	4.04	9.25	2.63	5.16
N21a	7.74	8.72	5.92	6.57	4.93	4.04	0	11.59	5.93	8.45
N21b	14.25	11.98	11.55	11.74	7.61	9.25	11.59	0	9.77	10.24
N22a	8.02	4.17	3.28	3.57	4.52	2.63	5.93	9.77	0	3.63
N22b	9.40	3.64	5.31	5.27	6.76	5.16	8.45	10.24	3.63	0

Table 16. Parameter estimates and individual standard errors for five two-variable linear regression relationships for each of the ten forms in the *fulva-rudis-texana* complex of *Aphaenogaster*. Estimators of the regression parameters are b_0 , the Y -intercept, and b_1 , the slope. For each form, standard errors are based on data from that form only.

Y	X	form	b_0	b_1	$SE(b_0)$	$SE(b_1)$
HL	HW	fulva	0.334	0.881	0.031	0.031
		N16	0.272	0.941	0.056	0.055
		N17	0.274	0.946	0.044	0.046
		N18	0.294	0.918	0.032	0.033
		N19	0.476	0.786	0.044	0.054
		N20	0.317	0.958	0.038	0.044
		N21a	0.437	0.835	0.150	0.160
		N21b	0.236	1.135	0.037	0.040
		N22a	0.408	0.833	0.025	0.028
		N22b	0.382	0.874	0.040	0.039
SL	HW	fulva	0.470	0.843	0.056	0.056
		N16	0.542	0.736	0.064	0.064
		N17	0.648	0.742	0.050	0.052
		N18	0.722	0.638	0.058	0.060
		N19	0.729	0.749	0.158	0.193
		N20	0.642	0.783	0.066	0.076
		N21a	0.624	0.841	0.246	0.262
		N21b	0.575	1.009	0.076	0.083
		N22a	0.668	0.724	0.044	0.049
		N22b	0.576	0.776	0.051	0.050
ISPL	WLA	fulva	-0.323	0.333	0.047	0.030
		N16	-0.120	0.162	0.062	0.039
		N17	-0.116	0.180	0.037	0.023
		N18	-0.086	0.158	0.041	0.025
		N19	-0.222	0.224	0.035	0.023
		N20	-0.148	0.184	0.024	0.015
		N21a	-0.380	0.353	0.093	0.056
		N21b	-0.091	0.105	0.044	0.026
		N22a	-0.192	0.211	0.032	0.020
		N22b	-0.253	0.238	0.037	0.022
FW	FL	fulva	-0.006	0.141	0.018	0.011
		N16	-0.006	0.170	0.034	0.022
		N17	-0.032	0.138	0.024	0.015
		N18	0.010	0.151	0.024	0.015
		N19	0.040	0.123	0.024	0.015
		N20	0.020	0.138	0.025	0.015
		N21a	0.096	0.091	0.054	0.032
		N21b	-0.057	0.178	0.021	0.012
		N22a	0.008	0.154	0.016	0.010
		N22b	0.055	0.130	0.026	0.016
EL	WLA	fulva	-0.016	0.160	0.026	0.016
		N16	-0.048	0.184	0.025	0.015
		N17	-0.035	0.172	0.020	0.012
		N18	-0.033	0.168	0.026	0.016
		N19	-0.126	0.237	0.059	0.039
		N20	-0.037	0.182	0.021	0.013
		N21a	-0.049	0.198	0.067	0.041
		N21b	-0.017	0.152	0.025	0.015
		N22a	-0.042	0.179	0.016	0.010
		N22b	0.007	0.146	0.038	0.023

Table 17. Sympatric occurrences (at the same locality, within 500m) of forms in the fulva-rudis-texana complex of Aphaenogaster found in the thesis research. See Table 2 for full locality data. All records in this list were verified karyotypically, and also electrophoretically where N22b was involved. Other cases of probable sympatry are discussed in the text.

<u>Pair of forms</u>	<u>Localities</u>
<u>fulva</u> , N19	MO2
<u>fulva</u> , N20	FL5, NC7, NC8, NJ1, SC1, SC2
<u>fulva</u> , N21a	FL2, GA4
<u>fulva</u> , N22a	IN2, KY1, MD1, NC1, NC4, NC11, NJ4, OH1
N16, N17	OH2, ON5, ON6
N16, N18	ON1
N16, N22a	NJ5
N16, N22b	ON6
N17, N18	ON3
N17, N22a	KY2
N19, N22a	MO1
N20, N22a	NC2, NJ1
N22a, N22b	IN3, MD3

Figure 1. Representative karyotypes for six forms in the fulva-rudis-texana complex of Aphaenogaster. The form code and/or species name, colony number, locality code (see Table 2), and chromosome number are listed. a. N16, A231, ON5, $n = 16$. b. N17, A204, ON5, $2n = 34$. c. N18 (picea), A223, ON3, $n = 18$. d. A. fulva, A154, NJ4, $n = 18$. e. N19, A377, MO2, $n = 19$. f. N20 (carolinensis), A272, NC8, $2n = 40$. Scale bars to the left of each karyotype represent $5\ \mu\text{m}$.

Figure 2. Representative karyotypes for four forms in the fulva-rudis-texana complex (a-d) and three outgroup species of Aphaenogaster. The form code and/or species name, colony number, locality code (see Table 2), and chromosome number are listed. a. N21a (miamiana), A249, FL1, $2n = 42$. b. N21b (texana), A422, TX1, $2n = 42$. c. N22a (rudis), A085, AL1, $2n = 44$. d. N22b, A218, ON6, $n = 22$. e. A. lamellidens, A340, KY1, $n = 19$. f. A. tennesseensis, A198, ON5, $2n = 26$. g. A. treatae, A215, ON5, $n = 21$. Scale bars to the left of each karyotype represent $5\ \mu\text{m}$.

a |

b |

c |

d |

e |

f |

g |

Figure 3. Karyotypes of colonies belonging to the fulva-rudis-texana complex in Aphaenogaster. Additional karyotypes for three forms (a-d) and three karyotypes of an N17 $\times$ N22b hybrid colony (e-g). The form code or species name, colony number, locality code (see Table 2), and chromosome number are listed. **a.** N20 (carolinensis), A179, NJ1, $2n = 40$. **b.** N22a (rudis), A174, NJ1, $2n = 44$. **c.** N22a (rudis), A125, NC11, $n = 22$. **d.** N22b, A222, ON6, $2n = 44$. **e-g.** N17 $\times$ N22b hybrid, A221, ON6. Karyotypes in **e, f** from same male brain, both $n = 18$. Karyotype in **g** from a different male brain, $n = 19$. Scale bars to the left of each karyotype represent $5 \mu\text{m}$.

a. | { 4 || i i j j j i i i j i
 i i i i j j j i i i j i j j

b. | 8 8 8 8 8 8 8 8 8 8 8 8
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c. | 8 8 8 8 8 8 8 8 8 8 8 8

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e. | { (j i j i i i i i i i

f. | {) { i i j i i i i i i i

g. | 8 8 8 i i i i i i i i i i

Figure 4. Metaphase plates of colonies belonging to the fulva-rudis-texana complex in Aphaenogaster. The form code and/or species name, colony number, locality code (see Table 2), tissue karyotyped, and chromosome number are listed. **a.** N17, A208, ON5, male brain, $n = 17$. Arrow points to a chromosome with a strong secondary constriction. **b.** A. fulva, A102, SC1, worker brain, $2n = 36$. Arrows point to submetacentrics characteristic of fulva karyotype. **c.** N18 (picea), A324, GA3, male brain, $n = 18$. **d-f.** N22a (rudis), KY1, testes from three males. Chromosome numbers are $n = 23$, $n = 24$, and $n = 28$, respectively, illustrating supernumerary chromosome variation for this usual $n = 22$ form. **g.** N22a (rudis), A334, TN2, worker brain, $2n = 46$. Arrows point to probable supernumerary chromosomes. **h.** N20 (carolinensis), A180, NJ1, queen brain, $4n = 80$. Note pairing of bivalents. Scale bar at bottom of **a** represents $5\ \mu\text{m}$; all other karyotypes in this figure at same scale.

Figure 5. Idiograms for haploid karyotypes prepared from the measurement data on the Aphaenogaster karyotypes illustrated in Figures 1a (N16), 1b (N17), 3e (N17 $\times$ N22b hybrid), 1c (N18, picea), and 1d (fulva). Vertical axis represents percentage relative length of haploid genome.

Figure 6. Idiograms for haploid karyotypes prepared from the measurement data on the Aphaenogaster karyotypes illustrated in Figures 1e (N19), 1f (N20, carolinensis), 2a (N21a, miamiana), and 2e (lamellidens). Vertical axis represents percentage relative length of haploid genome.

Figure 7. Idiograms for haploid karyotypes prepared from the measurement data on the Aphaenogaster karyotypes illustrated in Figures 2b (N21b, texana), 2c (N22a, rudis), 2d (N22b), 2f (tennesseensis), and 2g (treatae). Vertical axis represents percentage relative length of haploid genome.

Figure 8. a. Primary chromosomal rearrangements required to connect the basic karyotypes of six forms in the fulva-rudis-texana complex with $n \geq 19$. **b.** Speculative phylogeny of karyotype evolution in the fulva-rudis-texana complex, using the karyotype of Aphaenogaster tennesseensis as the "ancestral" karyotype. Events in karyotype evolution are: 1, centric fission of chromosome #5 of tennesseensis; 2, pericentric inversion of chromosome #1 of tennesseensis to centre centromere as in chromosome #1 of N19, and centric fissions of chromosomes #3–4, 6–8 of tennesseensis; 3, centric fissions of chromosomes #1–2 of N19; 4, subterminal fission (possible translocation) of chromosome #1 of N19 to create very small acrocentric; 5, centric fission of chromosome #2 of N20; 6, centric fission of chromosome #1 of N22b; 7, centric fissions of chromosomes #1–2 of tennesseensis; 8, at least two centric fissions and one pericentric inversion required (?); 9, centric fusion of chromosome #2 and a small acrocentric of N18 to create chromosome #1 of N17. The placement of fulva is uncertain. Polarity of character 4 supported by outgroup analysis, and centric fissions are assumed to predominate over centric fusions in accordance with the minimum interaction hypothesis.

N21b (texana)

↕ 2 centric fissions or fusions

N19

↕ subterminal fission or terminal fusion

N20 (carolinensis)

↕ centric fission or fusion

N21a (miamiana)

↕ centric fission or fusion

N22a (rudis), N22b

a

b

Figure 9. Zymograms representing banding positions for three enzyme systems in Aphaenogaster. a. Isocitrate dehydrogenase (IDH). Dotted lines represent light bands that were interpreted as artifact bands. (1) *Idh*^{1/1}. (2) *Idh*^{2/2}. (3) A221 hybrid, scored *Idh*^{1/2}. (4) Rare variant, colony A212 of N17. (5) Rare variant, colony A384 of N22a (rudis). (6) Rare variant, colony A375 of N19. (7) Possible variant, colony A392 of N22a (rudis). (8) A. tennesseensis.

b. Aspartate aminotransferase (AAT). (1) *Aar*^{1/1}. (2) *Aar*^{2/2}. (3) *Aar*^{1/2}. (4) Possible variant band found in three colonies of N16, appearing slightly slower than that of *Aar*^{1/1}. (5) Variant of A. lamellidens (colony A340), possible heterozygote of *Aar*¹ with an allele coding for a slightly slower enzyme when homozygous. (6) Variant banding pattern found in two colonies of N20 (carolinensis), poor resolution, but suggesting heterozygote of *Aar*² with a rare allele that when homozygous codes for an enzyme with intermediate mobility between those of *Aar*^{1/1} and *Aar*^{2/2}.

c. Phosphoglucumutase (PGM-1,2). (1)–(4) Some banding patterns for fulva: (1) *Pgm-1*^{2/2}, *Pgm-2*^{1/2}; (2) *Pgm-1*^{2/2}, *Pgm-2*^{2/3}; (3) Probable heterozygote of *Pgm-1*² with a rare, "slower" allele (found only in colony A167), *Pgm-2*^{1/4}; (4) *Pgm-1*^{2/2}, *Pgm-2*^{2/3} (or *Pgm-1*², *Pgm-2*³ if male). (5) A. tennesseensis, *Pgm-1*^{2/2?}, *Pgm-2*^{3/5}. (6) *Pgm-1*^{1/1}, *Pgm-2*^{1/7}. (7) *Pgm-1*^{1/1}, *Pgm-2*^{6/6}. (8) Banding pattern for N22b and A221 hybrid colony; extra slow band may be IDH.

Figure 10. Frontal views of worker heads of members of the fulva-rudis-texana complex in Aphaenogaster.. Data given represents form code and/or species name, colony number, locality code (see Table 2) or locality, head width (HW) in mm, and Weber's Length of Alitrunk (WLA) in mm. a. A. fulva, A168, NJ3, HW = 1.11, WLA = 1.78. b, c. A. fulva, A385, IN2; HW = 1.00, WLA = 1.62 and HW = 0.63, WLA = 1.21, respectively. d. N16, A188, ON5, HW = 0.99, WLA = 1.64. e. N17, A204, ON5, HW = 1.04, WLA = 1.77. f. N18 (picea), A203, ON1, HW = 1.05, WLA = 1.81. g. N16 (?), A380; MO: Franklin Co., Shaw Arboretum; HW = 0.76, WLA = 1.30. h. N19, A372, MO1, HW = 0.87, WLA = 1.65. i. N20 (carolinensis), A179, NJ1, HW = 1.00, WLA = 1.73.

Figure 11. Frontal views of worker heads of members of the fulva-rudis-texana complex in Aphaenogaster. Data given represents form code and/or species name, colony number, locality code (see Table 2) or locality, head width (HW) in mm, and Weber's Length of Alitrunk (WLA) in mm. a. N21a (miamiana), A096, FL3, HW = 0.87, WLA = 1.64. b. N21a (miamiana), A242, FL1, HW = 1.00, WLA = 1.77. c. N21b (?) (?texana), GJU0327; TX: Brownsville; HW = 0.97, WLA = 1.83. d. N21b (texana), GJU1046; AZ: Cochise Co., Chiricahua National Monument, Herb Martyr Forest Camp, 6150', HW = 1.00, WLA = 1.87. e. N21b, A437 (texana), AZ1, HW = 0.71, WLA = 1.40. f. A. punctaticeps, unique paratype, NM: Dona Ana Co., 45 km NE Las Cruces, Jornada Experimental Range, HW = 1.04, WLA = 1.90. g. N22a (rudis), A085, AL1, HW = 0.83, WLA = 1.49. h. N22b, A222, ON6, HW = 0.95, WLA = 1.62. i. N17 × N22b hybrid, A221, ON6, HW = 1.04, WLA = 1.77.

Figure 12. Lateral views of worker alitrunks of members of the fulva-rudis-texana complex in Aphaenogaster. Individuals illustrated are the same illustrated in Fig. 10a-f,h,i, respectively. Data given represents form code and/or species name, colony number, locality code (see Table 2) or locality, and Weber's Length of Alitrunk (WLA) in mm. a. A. fulva, A168, NJ3, WLA = 1.78. b, c. A. fulva, A385, IN2; WLA = 1.62 and WLA = 1.21, respectively. d. N16, A188, ON5, WLA = 1.64. e. N17, A204, ON5, WLA = 1.77. f. N18 (picea), A203, ON1, WLA = 1.81. g. N19, A372, MO1, WLA = 1.65. h. N20 (carolinensis), A179, NJ1, WLA = 1.73.

Figure 13. Lateral views of worker alitrunks of members of the fulva-rudis-texana complex in Aphaenogaster. Individuals illustrated are the same illustrated in Fig. 11a-d,f-i, respectively. Data given represents form code and/or species name, colony number, locality code (see Table 2) or locality, and Weber's Length of Alitrunk (WLA) in mm. a. N21a (miamiana), A096, FL3, WLA = 1.64. b. N21a (miamiana), A242, FL1, WLA = 1.77. c. N21b (?) (?texana), GJU0327; TX: Brownsville; WLA = 1.83. d. N21b (texana), GJU1046; AZ: Cochise Co., Chiricahua National Monument, Herb Martyr Forest Camp, 6150', WLA = 1.90. e. A. punctaticeps, unique paratype, NM: Dona Ana Co., 45 km NE Las Cruces, Jornada Experimental Range, WLA = 1.90. f. N22a (rudis), A085, AL1, WLA = 1.49. g. N22b, A222, ON6, WLA = 1.62. h. N17 × N22b hybrid, A221, ON6, WLA = 1.77.

Figure 14. Lateral view of the mesopleurae (mesothoracic sternite and episternite) and surrounding area of the alitrunk on queens of the fulva-rudis-texana complex of Aphaenogaster. Data given represents form code and/or species name, colony number, and locality code (see Table 2). a. fulva, A385, IN2. b. N16, A409, ON5. c. N18 (picea), A365, PA4. d. N20 (carolinensis), A311, GA1.

Figure 15. Plot of the first two canonical variates, from the canonical variate analysis of the log transformed regular data set with all forms of the fulva-rudis-texana complex in Aphaenogaster included. The small graphs in b-e contain subsets of the forms, in order to give a breakdown of the graph in a with all forms plotted. Form codes for which names can be assigned are: N18 (picea), N20 (carolinensis), N21a (miamiana), N21b (texana), N22a (rudis).

a

b

c

d

e

Figure 16. Plots utilizing the first three canonical variates from the canonical variate analysis of the log transformed regular data set of the fulva-rudis-texana complex in Aphaenogaster, with fulva and N21b deleted from the analysis. **a.** Canonical Variate 2 on Canonical Variate 1. **b.** Canonical Variate 3 on Canonical Variate 1. Form codes for which names can be assigned are: N18 (pacea), N20 (carolinensis), N21a (miamiana), N21b (texana), N22a (rudis).

a

b

Figure 17. Plots of the two canonical variates from canonical variate analyses utilizing only the data on selected subsets of three forms from the log transformed regular data set of the fulva-rudis-texana complex in Aphaenogaster. a. N16, N22a (rudis), N22b. b. N17, N18 (picea), N22a (rudis).

a

b

Figure 18. a. Plot of the two canonical variates from a canonical variate analysis utilizing only the data on N20 (carolinensis), N21a (miamiana), and N22a (rudis), from the log transformed regular data set of the fulva-rudis-texana complex in Aphaenogaster. b. Plot of the first two canonical variates, from the canonical variate analysis of the log transformed regular data set with all forms of the fulva-rudis-texana complex of Aphaenogaster included; this figure is the same as Fig. 15a, but an additional 30 data points have been plotted. These data points represent additional measured workers for which canonical variates were calculated using the functions generated by the canonical analysis on the log transformed regular data set. Legend: as for Fig. 15a, but larger symbols represent the additional individuals (identifications for these individuals were made prior to the multivariate analysis). Two additional symbols represent a huachucana paratype (large open diamond with a small dot inside, near right border) and the punctaticeps paratype (large open circle with a dot inside, near top border). Since the large solid inverted triangle representing Crozier's voucher specimen has been largely obscured by the other fulva symbols, a small white dot has been placed on top of it. Letters (A, B, C) represent specific individuals referred to in the text.

a

b

Figure 19. Scattergrams (or graph of regression lines only) of HL (head length) on HW (head width) with fitted regression lines, for forms in the fulva-rudis-texana complex of Aphaenogaster.
a, all forms; **b**, N16, N20, N21b; **c**, regression lines only for all forms; **d**, fulva and N16; **e**, N17 and N18; **f**, N19, N20, N21a, N21b.

a

b

c

d

e

f

Figure 20. Two-variable scattergrams with fitted regression lines, for forms in the fulva-rudis-texana complex of Aphaenogaster. a, HL (head length) on HW (head width) for N20, N22a; b, HL on HW for N22a, N22b; c, SL (scape length) on HW for all forms; d, SL on HW for N16, N20, N21b; e, SL on HW for fulva, N21a, N21b; f, SL on HW for fulva, N20, N22a. Form codes for which names can be assigned are: N18 (picea), N20 (carolinensis), N21a (miamiana), N21b (texana), N22a (rudis).

a

b

c

d

e

f

Figure 21. Scattergrams of SL (scape length) on HW (head width) with fitted regression lines, for forms in the fulva-rudis-texana complex of Aphaenogaster. a, fulva, N16; b, N16, N22a, N22b; c, N19, N20; d, N17, N22a; e, N16, N17, N18; f, all forms except fulva. Form codes for which names can be assigned are: N18 (picea), N20 (carolinensis), N21a (miamiana), N21b (texana), N22a (rudis).

a

b

c

d

e

f

Figure 22. Two-variable scattergrams with fitted regression lines, for forms in the fulva-rudis-texana complex of Aphaenogaster. **a**, ISPL (interspinal length) on WLA (Weber's length of alitrunk) for all forms; **b**, ISPL on TL (tibial length) for all forms; **c**, ISPL on WLA for N19, N20, N21a, N21b; **d**, ISPL on WLA for N16, N17, N18, N22b; **e**, ISPL on TL for N17, N18, N22a; **f**, FW (femur width) on FL (femur length) for all forms. Form codes for which names can be assigned are: N18 (picea), N20 (carolinensis), N21a (miamiana), N21b (texana), N22a (rudis).

a

b

c

d

e

f

Figure 23. Two-variable scattergrams with fitted regression lines, for forms in the fulva-rudis-texana complex of Aphaenogaster. a, FW (femur width) on FL (femur length) for fulva, N16; b, EL (eye length) on WLA (Weber's length of alitrunk) for all forms; c, EL on WLA for N17, N18, N21a; d, EL on HW (head width) for N17, N18, N21a; e, EL on WLA for N20, N21a, N21b; f, CI (cephalic index) on WLA for all forms. Form codes for which names can be assigned are: N18 (picea), N20 (carolinensis), N21a (miamiana), N21b (texana), N22a (rudis).

a

b

c

d

e

f

Figure 24. Two-variable scattergrams involving indices for forms in the fulva-rudis-texana complex of Aphaenogaster. Each scattergram includes plots for all forms. In **a-d** an index has been plotted on WLA (Weber's length of alitrunk) and regression lines have been fitted. The Y-axis variables are: **a**, SI1 (scape index 1); **b**, SPI (spinal index); **c**, EI (eye, or ocular, index); **d**, FI1 (femur index 1). Both variables are indices in **e-f**: **e**, EI on FI1; **f**, SPI on FI1. Form codes for which names can be assigned are: N18 (picea), N20 (carolinensis), N21a (miamiana), N21b (texana), N22a (rudis).

a

b

c

d

e

f

Map 1. Localities in eastern North America from which one or more Aphaenogaster colonies were karyotyped (localities from Arizona and Texas not illustrated). See Table 2 for detailed locality information.

Map 2. Locality records for karyotyped colonies of Aphaenogaster: A. fulva (solid circles), N16 (open circles).

Map 3. Locality records for karyotyped colonies of Aphaenogaster: N17 (solid inverted triangles), N22a (rudis, solid circles).

Map 4. Locality records for karyotyped colonies of Aphaenogaster: N18 (picea, solid circles), N19 (open squares), N20 (carolinensis, solid squares), N21a (miamiana, open circles), N22b (solid triangles).

References

- Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. U.S. Department of Commerce, NOAA Technical Report NMFS 61.
- Agosti, D., and C. A. Collingwood. 1987a. A provisional list of the Balkan ants (Hym., Formicidae) and a key to the worker caste. I. Synonymic list. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 60: 51–62.
- Agosti, D., and C. A. Collingwood. 1987b. A provisional list of the Balkan ants (Hym. Formicidae) with a key to the worker caste. II. Key to the worker caste, including the European species without the Iberian. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 60: 261–293.
- Angus, R. B. 1982. Separation of two species standing as Helophorus aquaticus (L.) (Coleoptera, Hydrophilidae) by banded chromosome analysis. *Systematic Entomology* 7: 265–281.
- Angus, R. B. 1983. Separation of Helophorus grandis, maritimus and occidentalis sp.n. (Coleoptera, Hydrophilidae) by banded chromosome analysis. *Systematic Entomology* 8: 1–13.
- Atchley, W. R., C. T. Gaskins, and D. Anderson. 1976. Statistical properties of ratios. I. Empirical results. *Systematic Zoology* 25: 137–148.
- Baker, R. J., W. R. Atchley, and V. R. McDaniel. 1972. Karyology and morphometrics of Peters' tent-making bat, Uroderma bilobatum Peters (Chiroptera, Phyllostomatidae). *Systematic Zoology* 21: 414–429.
- Baker, R. J., M. B. Qumsiyeh, and C. S. Hood. 1987. Role of chromosomal banding patterns in understanding mammalian evolution. In *Current Mammalogy*, ed. H. H. Genoways, pp. 67–96. New York: Plenum.
- Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16: 113–148.
- Beattie, A. J. 1985. *The Evolutionary Ecology of Ant-Plant Mutualisms*. New York: Cambridge University Press.
- Bhatkar, A., and W. H. Whitcomb. 1970. Artificial diet for rearing various species of ants. *Florida Entomologist* 53: 229–232.
- Bigelow, R. S. 1965. Hybrid zones and reproductive isolation. *Evolution* 19: 449–458.
- Blackith, R. E., and R. A. Reyment. 1971. *Multivariate Morphometrics*. London: Academic Press.

- Bogart, J. P., and M. Tandy. 1981. Chromosome lineages in African ranoid frogs. *Monitore Zoologico Italiano (N.S.)* 15: 55–91.
- Bolton, B. 1982. Afrotropical species of the myrmicine ant genera Cardiocondyla, Leptothorax, Melissotarsus, Messor and Cataulacus (Formicidae). *Bulletin of the British Museum (Natural History), Entomology* 45: 307–370.
- Bolton, B. 1991. New myrmicine ant genera from the Oriental Region (Hymenoptera: Formicidae). *Systematic Entomology* 16: 1–13.
- Bookstein, F., B. Chernoff, R. Elder, J. Humphries, G. Smith, and R. Strauss. 1985. *Morphometrics in Evolutionary Biology*. Special Publication 15, The Academy of Natural Sciences of Philadelphia.
- Boomsma, J. J. 1990. Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. *Evolution* 44: 1026–1034.
- Boomsma, J. J., and A. Grafen. 1990. Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. *Evolution* 44: 1026–1034.
- Boomsma, J. J., and J. A. Isaaks. 1985. Energy investment and respiration in queens and males of Lasius niger (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* 18: 19–27.
- Bourke, A. F. G. 1988. Worker reproduction in the higher eusocial Hymenoptera. *Quarterly Review of Biology* 63: 291–311.
- Bourke, A. F. G., and N. R. Franks. 1991. Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. *Biological Journal of the Linnean Society* 43: 157–178.
- Brown, W. L., Jr., and E. O. Wilson. 1954. The case against the trinomen. *Systematic Zoology* 3: 174–176.
- Buckley, S. B. 1867. Descriptions of new species of North American Formicidae. *Proceedings of the Entomological Society of Philadelphia* 6: 152–172, 335–350.
- Bullini, L., and G. Nascetti. 1990. Speciation by hybridization in phasmids and other insects. *Canadian Journal of Zoology* 68: 1747–1760.
- Buren, W. F. 1944. A list of Iowa ants. *Iowa State College Journal of Science* 18: 277–312.
- Cagniant, H. 1968. Description d'Aphaenogaster crocea faureli n. subsp. d'Algérie. *Bulletin de la Societe Entomologique de France* 73: 232–235.
- Cagniant, H. 1982. Contribution à la connaissance des fourmis marocaines. Aphaenogaster dejeani, nov. sp. (Hyménoptères, Formicoidea, Myrmicidae). *Nouvelle Revue d'Entomologie* 12: 281–286.

- Cagniant, H. 1984. Contribution à la connaissance des fourmis marocaines: Aphaenogaster espadeleri n. sp. (Hymenoptera, Myrmicidae). Nouvelle Revue d'Entomologie (N.S.) 1: 387-395.
- Cagniant, H. 1987. Contribution à la connaissance des fourmis marocaines. Nouvelle description et compléments à la définition de l'espèce Aphaenogaster praedo Emery (Hyménoptères, Formicoidea, Myrmicidae). Problèmes posés par le groupe "praedo". Bulletin de la Société d'Histoire Naturelle de Toulouse 123: 159-165.
- Cagniant, H. 1988a. Contribution à la connaissance des fourmis marocaines. Description des trois castes d'Aphaenogaster wilsoni n. sp. (Hymenoptera, Myrmicidae). Nouvelle Revue d'Entomologie (N.S.) 5: 49-55.
- Cagniant, H. 1988b. Contribution à la connaissance des fourmis marocaines. Description des trois castes d'Aphaenogaster torossiani n. sp. et notes biologiques. Bulletin de la Société Entomologique de France 92: 241-250.
- Cagniant, H. 1988c. Contribution à la connaissance des fourmis marocaines: Aphaenogaster baronii n. sp. (Hyménoptères, Formicoidea, Myrmicidae). Bulletin de la Société d'Histoire Naturelle de Toulouse 124: 43-50.
- Cagniant, H. 1989a. Contribution à la connaissance des fourmis marocaines Aphaenogaster gemella au Maroc: Nouvelle description d'Aphaenogaster gemella ssp. marocana Forel (n. status) de la région de Tanger. Problèmes biogéographiques soulevés par l'espèce Aphaenogaster gemella (Roger) (Hyménoptères, Formicoidea, Myrmicidae). Bulletin de la Société d'Histoire Naturelle de Toulouse 125: 47-54.
- Cagniant, H. 1989b. Contribution à la connaissance des fourmis marocaines. Description des trois castes d'Aphaenogaster weulersseae n. sp.; notes biologiques et écologiques; étude comparée de trois populations [Hym. Formicoidea Myrmicidae]. Bulletin de la Société Entomologique de France 94: 113-125.
- Carpenter, J. M. 1990. Of genetic distances and social wasps. Systematic Zoology 39: 391-397.
- Carroll, J. F. 1975. Biology and ecology of ants of the genus Aphaenogaster in Florida. PhD thesis, University of Florida, Gainesville, FL.
- Carroll, J. F., J. W. Kimbrough, and W. H. Whitcomb. 1981. Mycophagy by Aphaenogaster spp. (Hymenoptera: Formicidae). Proceedings of the Entomological Society of Washington 83: 326-331.
- Carson, H. L., P. S. Nair, and F. M. Sene. 1975. Drosophila hybrids in nature: proof of gene exchange between sympatric species. Science 189: 806-807.
- Carter, W. G. 1962a. Ants of the North Carolina Piedmont. Journal of the Elisha Mitchell Scientific Society 78: 1-18.

- Carter, W. G. 1962b. Ant distribution in North Carolina. *Journal of the Elisha Mitchell Scientific Society* 78: 150–204.
- Cheliak, W. M., and J. A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42, Petawawa National Forestry Institute, Canadian Forestry Service.
- Cokendolpher, J. C., and J. D. Brown. 1985. Air-dry method for studying chromosomes of insects and arachnids. *Entomological News* 96: 114–118.
- Cole, A. C., Jr. 1938. Suggestions concerning taxonomic nomenclature of the hymenopterous family Formicidae, and descriptions of three new ants. *American Midland Naturalist* 19: 236–241.
- Cole, A. C., Jr. 1940. A guide to the ants of the Great Smoky Mountains National Park, Tennessee. *American Midland Naturalist* 24: 1–88.
- Cole, A. C., Jr. 1953. Studies of New Mexico ants. II. A description of a new subspecies of Aphaenogaster huachucana (Hymenoptera: Formicidae). *Journal of the Tennessee Academy of Science* 28: 82–84.
- Cole, A. C., Jr. 1968. Pogonomyrmex harvester ants: a study of the genus in North America. Knoxville, TN: University of Tennessee Press.
- Collingwood, C. A. 1978. A provisional list of Iberian Formicidae with a key to the worker caste. *Eos* 52: 65–95.
- Creighton, W. S. 1934. Descriptions of three new North American ants with certain ecological observations on previously described forms. *Psyche* 41: 185–210.
- Creighton, W. S. 1950. The ants of North America. *Bulletin of the Museum of Comparative Zoology* 104: 1–585 + 57 pl.
- Creighton, W. S. 1951. Studies on Arizona ants. 2. New data on the ecology of Aphaenogaster huachucana and a description of the sexual forms. *Psyche* 58: 89–99.
- Crosland, M. W. J., R. H. Crozier, and H. T. Imai. 1988. Evidence for several sibling biological species centred on Myrmecia pilosula (F. Smith) (Hymenoptera: Formicidae). *Journal of the Australian Entomological Society* 27: 13–14.
- Crozier, R. H. 1968. An acetic acid dissociation, air-drying technique for insect chromosomes, with aceto-lactic orcein staining. *Stain Technology* 43: 171–173.
- Crozier, R. H. 1970. Pericentric rearrangement polymorphism in a North American dolichoderine ant (Hymenoptera: Formicidae). *Canadian Journal of Genetics and Cytology* 12: 541–546.

- Crozier, R. H. 1974. Allozyme analysis of reproductive strategy in the ant Aphaenogaster rudis. *Isozyme Bulletin* 7: 18.
- Crozier, R. H. 1975. Hymenoptera: Animal Cytogenetics. Vol. 3. *Insecta* 7. Berlin: Gebruder Borntrager.
- Crozier, R. H. 1977. Genetic differentiation between populations of the ant Aphaenogaster 'rudis' in the southeastern United States. *Genetica* 47: 17–36.
- Crozier, R. H. 1981. Genetic aspects of ant evolution. *In* *Evolution and Speciation, Essays in Honour of M. J. D. White*, ed. W. R. Atchley and D. Woodruff, pp. 356–370. Cambridge: Cambridge University Press.
- Crozier, R. H., P. Pamilo, R. W. Taylor, and Y. C. Crozier. 1986. Evolutionary patterns in some Australian species in the ant genus Rhytidoponera. *Australian Journal of Zoology* 34: 535–560.
- Daly, H. V., and S. S. Balling. 1978. Identification of Africanized honeybees in the Western Hemisphere by discriminant analysis. *Journal of the Kansas Entomological Society* 51: 857–869.
- Dawkins, R. 1989. *The Selfish Gene*, new edition. Oxford: Oxford University Press.
- Dennis, C. A. 1938. The distribution of ant species in Tennessee with reference to ecological factors. *Annals of the Entomological Society of America* 31: 267–308.
- Deyrup, M., and J. Trager. 1986. Ants of the Archbold Biological Station, Highlands County, Florida (Hymenoptera: Formicidae). *Florida Entomologist* 69: 206–228.
- Deyrup, M. A., N. Carlin, J. Trager, and G. Umphrey. 1988. A review of the ants of the Florida Keys. *Florida Entomologist* 71: 163–176.
- Deyrup, M., C. Johnson, G. C. Wheeler, and J. Wheeler. 1989. A preliminary list of the ants of Florida. *Florida Entomologist* 72: 91–101.
- DuBois, M. B., and W. E. LaBerge. 1988. Annotated list of ants in Illinois (Hymenoptera: Formicidae). *In* *Advances in Myrmecology*, ed. J. Trager, pp. 133–156. New York: E. J. Brill.
- Elmes, G. W. 1978. A morphometric comparison of three closely related species of Myrmica (Formicidae), including a new species from England. *Systematic Entomology* 3: 131–145.
- Emery, C. 1895. Beiträge zur Kenntniss der nordamerikanischen Ameisenfauna. *Zoologischen Jahrbüchern, Abtheilung für Systematik, Geographie und Biologie der Thiere* 8: 257–360.
- Emery, C. 1921. Hym. fam. Formicidae subfam. Myrmicinae. *In* *Genera Insectorum*, ed. P. A. G. Wytsman, fasc. 174a: 1–94.

- Enzmann, J. 1947. New forms of Aphaenogaster and Novomessor. Journal of the New York Entomological Society 55: 147–153.
- Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Great Britain: Oxford University Press.
- Fisher, R. A. 1936. The use of multiple measurements in taxonomic problems. Annals of Eugenics 7: 179–188.
- Francoeur, A. 1973. Révision taxonomique des espèces néarctiques du groupe fusca, genre Formica. Mémoires de la Société Entomologique du Québec 3: 1–316.
- Futuyma, D. J. 1986. Evolutionary Biology, 2nd edition. Sunderland, MA: Sinauer.
- Goodman, L. A. 1965. On simultaneous confidence intervals for multinomial proportions. Technometrics 7: 247–254.
- Green, D. M., J. P. Bogart, and E. H. Anthony. 1980. An interactive, microcomputer-based karyotype analysis system for phylogenetic cytotaxonomy. Computers in Biology and Medicine 10: 219–227.
- Gregg, R. E. 1963. The Ants of Colorado. Denver: University of Colorado Press.
- Halliday, R. B. 1975. Electrophoretic variation of amylase in meat ants, Iridomyrmex purpureus, and its taxonomic significance. Australian Journal of Zoology 23: 271–276.
- Halliday, R. B. 1979. Esterase variations at three loci in meat ants. Journal of Heredity 70: 57–61.
- Halliday, R. B. 1981. Heterozygosity and genetic distance in sibling species of meat ants (Iridomyrmex purpureus group). Evolution 35: 234–242.
- Hamilton, W. D. 1964a. The genetical evolution of social behaviour. I. Journal of Theoretical Biology 7: 1–16.
- Hamilton, W. D. 1964b. The genetical evolution of social behaviour. II. Journal of Theoretical Biology 7: 17–52.
- Harrison, R. G., and D. M. Rand. 1989. Mosaic hybrid zones and the nature of species boundaries. In Speciation and Its Consequences, ed. D. Otte and J. A. Endler, pp. 111–133. Sunderland, MA: Sinauer.
- Hauschteck, E. 1962. Die Chromosomen einiger in der Schweiz vorkommender Ameisenarten. Vierteljahresschr. Naturforsch. Ges. Zur. 107: 213–222. [cited by Hung et al., 1972]
- Hauschteck-Jungen, E., and H. Jungen. 1983. Ant chromosomes. II. Karyotypes of western Palearctic species. Insectes Sociaux 30: 149–164.

- Headley, A. E. 1949. A population study of the ant Aphaenogaster fulva ssp. aquia Buckley (Hymenoptera, Formicidae). *Annals of the Entomological Society of America* 42: 265–272.
- Hebert, P. D. N., and M. J. Beaton. 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Handbook (32 pp.) publ. by Helena Laboratories, Beaumont, TX.
- Heinze, J. 1989. A biochemical approach toward the systematics of the Leptothorax "muscorum" group in North America (Hymenoptera: Formicidae). *Biochemical Systematics and Ecology* 17: 595–601.
- Heinze, J., and A. Buschinger. 1988. Electrophoretic variability of esterases in the ant tribe, Leptothoracini. *Biochemical Systematics and Ecology* 16: 217–221.
- Helms, K. R., and S. W. Rissing. 1990. Single sex alate production by colonies of Pheidole desertorum and Pheidole xerophila tucsonica (Hymenoptera: Formicidae). *Psyche* 97: 213–216.
- Hewitt, G. M. 1989. The subdivision of species by hybrid zones. In *Speciation and Its Consequences*, ed. D. Otte and J. A. Endler, pp. 85–110. Sunderland, MA: Sinauer.
- Hölldobler, B., and C. J. Lumsden. 1980. Territorial strategies in ants. *Science* 210: 732–739.
- Hölldobler, B., and E. O. Wilson. 1990. *The Ants*. Cambridge, MA: Belknap Press of Harvard University Press.
- Holmquist, G. P. 1989. Evolution of chromosome bands: molecular ecology of noncoding DNA. *Journal of Molecular Evolution* 28: 469–486.
- Hubbs, C. L. 1955. Hybridization between fish species in nature. *Systematic Zoology* 4: 1–20.
- Hung, A. C. F. 1985. Isozymes of two fire ant species and their hybrid. *Biochemical Systematics and Ecology* 13: 337–339.
- Hung, A. C. F., and S. B. Vinson. 1977. NAD-malate dehydrogenase isozyme patterns of interspecific hybrids in fire ants (Solenopsis spp.). *Isozyme Bulletin* 10: 30.
- Hung, A. C. F., H. T. Imai, and M. Kubota. 1972. The chromosomes of nine ant species (Hymenoptera: Formicidae) from Taiwan, Republic of China. *Annals of the Entomological Society of America* 65: 1023–1025.
- Imai, H. T. 1966. The chromosome observation techniques of ants and the chromosomes of Formicinae and Myrmicinae. *Acta Hymenopterologica* 2: 119–131.

- Imai, H. T. 1971. Karyological studies of Japanese ants. II. Species differentiation in Aphaenogaster; with special regard to their morphology, distribution and chromosomes. *Mushi* 44: 137–151 + 3 pl.
- Imai, H. T. 1974. B-chromosomes in the myrmicine ant, Leptothorax spinosior. *Chromosoma* (Berlin) 45: 431–444.
- Imai, H. T. 1975. Evidence for non-random localization of the centromere on mammalian chromosomes. *Journal of Theoretical Biology* 49: 111–123.
- Imai, H. T. 1976. Further evidence and biological significance for non-random localization of the centromere on mammalian chromosomes. *Journal of Theoretical Biology* 61: 195–203.
- Imai, H. T., and R. W. Taylor. 1989. Chromosomal polymorphisms involving telomere fusion, centromeric inactivation and centromere shift in the ant Myrmecia (pilosula) $n = 1$. *Chromosoma* (Berlin) 98: 456–460.
- Imai, H. T., and T. H. Yosida. 1965a. Chromosomes of male ants Aphaenogaster osimensis produced by parthenogenesis of workers. Annual Report of the National Institute of Genetics No. 16, pp. 53–54.
- Imai, H. T., and T. H. Yosida. 1965b. Polyploid cells observed in male and queen ants of Aphaenogaster osimensis. Annual Report of the National Institute of Genetics No. 16, p. 54.
- Imai, H. T., R. H. Crozier, and R. W. Taylor. 1977. Karyotype evolution in Australian ants. *Chromosoma* (Berlin) 59: 341–393.
- Imai, H. T., W. L. Brown, Jr., M. Kubota, H. S. Yong, and Y. P. Tho. 1984a. Chromosome observations on tropical ants from western Malaysia. II. Annual Report of the National Institute of Genetics No. 34, pp. 66–69.
- Imai, H. T., C. Baroni Urbani, M. Kubota, G. P. Sharma, M. N. Narasimhanna, B. C. Das, A. K. Sharma, A. Sharma, G. B. Deodikar, V. G. Vaidya, and M. R. Rajasekarasetty. 1984b. Karyological survey of Indian ants. *Japanese Journal of Genetics* 59: 1–32.
- Imai, H. T., T. Maruyama, T. Gojobori, Y. Inoue, and R. H. Crozier. 1986. Theoretical bases for karyotype evolution. I. The minimum-interaction hypothesis. *American Naturalist* 128: 900–920.
- Imai, H. T., R. W. Taylor, M. W. J. Crosland, and R. H. Crozier. 1988a. Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. *Japanese Journal of Genetics* 63: 159–185.
- Imai, H. T., R. W. Taylor, R. H. Crozier, M. W. L. Crosland, and G. P. Browning. 1988b. Chromosomal polymorphism in the ant Myrmecia (pilosula) $n = 1$. Annual Report of the National Institute of Genetics (Japan) No. 38, 1987, pp. 82–84.

- Imai, H. T., R. W. Taylor, M. Kubota, K. Ogata, and M. Y. Wada. 1990. Notes on the remarkable karyology of the primitive ant Nothomyrmecia macrops, and of the related genus Myrmecia (Hymenoptera: Formicidae). *Psyche* 97: 133–140.
- Jansen, D. H. 1967. Interaction of the bull's-horn acacia (Acacia cornigera L.) with an ant inhabitant (Pseudomyrmex ferruginea F. Smith) in eastern Mexico. *University of Kansas Science Bulletin* 47: 315–558.
- Jessen, K., and M. Klinkicht. 1990. Hybridization in the social parasitic ant genus Epimyrma (Hymenoptera, Formicidae). *Insectes Sociaux* 37: 273–293.
- Johnson, C. 1986. A north Florida ant fauna (Hymenoptera: Formicidae). *Insecta Mundi* 1: 243–246.
- Johnson, N. L., and S. Kotz. 1969. *Distributions in Statistics: Discrete Distributions*. New York: Wiley.
- Jones, R. N., and H. Rees. 1982. *B Chromosomes*. London: Academic Press.
- Key, K. H. L. 1981. Species, parapatry, and the morabine grasshoppers. *Systematic Zoology* 30: 425–458.
- Lachenbruch, P. A. 1975. *Discriminant Analysis*. New York: Hafner Press.
- Lachenbruch, P. A. 1982. Discriminant analysis. In *Encyclopedia of Statistical Sciences*, Vol. 2, ed. S. Kotz and N. L. Johnson, pp. 389–397. New York: Wiley.
- Lester, D. S., R. H. Crozier, and E. Shipp. 1979. G-banding patterns of the housefly, Musca domestica, autosomes and sex chromosomes. *Experientia* 35: 174.
- Levan, A., K. Fredga, and A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220.
- Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a source of variation for adaptation to new environments. *Evolution* 20: 315–336.
- Loiselle, R., A. Francoeur, K. Fischer, and A. Buschinger. 1990. Variations and taxonomic significance of the chromosome numbers in the nearctic species of the genus Leptothorax (s.s.) (Formicidae: Hymenoptera). *Caryologia* 43: 321–334.
- Lubischew, A. A. 1962. On the use of discriminant functions in taxonomy. *Biometrics* 18: 455–477.
- Macgregor, H., and J. Varley. 1988. *Working with Animal Chromosomes*, 2nd edition. New York: Wiley.
- MacKay, W. P. 1989. A new Aphaenogaster (Hymenoptera: Formicidae) from southern New Mexico. *Journal of the New York Entomological Society* 97: 47–49.

- Massaro, E. J. 1967. Induction of subunit reassociation among lactate dehydrogenase isozymes. *SABCO (Society for the Areas of Biological and Chemical Overlap) Journal (Osaka)* 3: 51–62.
- May, B. 1980. Starch gel isozyme electrophoresis: a methods manual. Extracted from *The Salmonid Genome: Evolutionary Restructuring Following a Tetraploid Event*, Ph.D. thesis, The Pennsylvania State University.
- May, B., J. E. Marsden, and C. G. Schenck. 1988. Electrophoretic procedures, recipes, and nomenclature used in the Cornell Laboratory for Ecological and Evolutionary Genetics. Ithaca, NY: CLEEG.
- Maynard Smith, J. 1982. *Evolution and the Theory of Games*. Cambridge, Great Britain: Cambridge University Press.
- Mayr, E. 1942. *Systematics and the Origin of Species*. New York: Columbia University Press.
- Mayr, E. 1963. *Animal Species and Evolution*. Cambridge, MA: Harvard University Press.
- Mayr, E. 1969. *Principles of Systematic Zoology*. New York: McGraw-Hill.
- Mayr, E. 1982. *The Growth of Biological Thought*. Cambridge, MA: Belknap Press of Harvard University Press.
- Mayr, G. L. 1853. Beiträge zur Kenntniss der Ameisen. *Verhandlungen des zoologisch-botanischen Vereins in Wien* 3: 101–114.
- Mehlhop, P., And A. L. Gardner. 1982. A rapid field technique for preparing ant chromosomes for karyotypic analysis. *Stain Technology* 57: 99–101.
- Menozzi, C. 1929. Una nuova specie di formica del genere *Aphaenogaster* Mayr del Nord America. *Bolletino del Laboratorio di Zoologia Generale e Agraria, Portici* 22: 282–284.
- Moody, J. V., and O. F. Francke. 1982. *The Ants (Hymenoptera, Formicidae) of Western Texas. Part 1: Subfamily Myrmicinae*. Lubbock, TX: Texas Tech Press.
- Nonacs, P. 1986a. Ant reproductive strategies and sex allocation theory. *Quarterly Review of Biology* 61: 1–21.
- Nonacs, P. 1986b. Sex-ratio determination within colonies of ants. *Evolution* 40: 199–204.
- Nuhn, T. P., and C. G. Wright. 1979. An ecological survey of ants (Hymenoptera: Formicidae) in a landscaped suburban habitat. *American Midland Naturalist* 102: 353–362.
- Nur, U. 1963. A mitotically unstable supernumerary chromosome with an accumulation mechanism in a grasshopper. *Chromosoma (Berlin)* 14: 407–422.

- Otte, D., and J. A. Endler, eds. 1989. *Speciation and Its Consequences*. Sunderland, MA: Sinauer.
- Palomeque, T., E. Chica, M. A. Cano, R. Díaz de la Guardia, and A. Tinaut. 1987. Cytogenetic studies in the genera Pheidole and Tetramorium (Hymenoptera, Formicidae, Myrmicinae). *Caryologia* 41: 289–298.
- Pearson, B. 1982. The taxonomic status of morphologically-anomalous ants in the Lasius niger/Lasius alienus taxon. *Insectes Sociaux* 29: 95–101.
- Pearson, B. 1983a. The classification of morphologically intermediate ants in the Lasius alienus/Lasius niger taxon. *Insectes Sociaux* 30: 100–105.
- Pearson, B. 1983b. Hybridisation between the ant species Lasius niger and Lasius alienus: the genetic evidence. *Insectes Sociaux* 30: 402–411.
- Pearson, B. 1987. The sex ratio of heathland populations of the ants Lasius alienus, Lasius niger and their hybrids. *Insectes Sociaux* 34: 194–203.
- Pearson, B., and A. R. Child. 1980. The distribution of an esterase polymorphism in macrogynes and microgynes of Myrmica rubra Latreille. *Evolution* 34: 105–109.
- Pontin, A. J. 1961. Population stabilization and competition between the ants Lasius flavus (F.) and L. niger (L.). *Journal of Animal Ecology* 30: 47–54.
- Potts, B. M., and J. B. Reid. 1988. Hybridization as a dispersal mechanism. *Evolution* 42: 1245–1255.
- Potts, B. M., and J. B. Reid. 1990. The evolutionary significance of hybridization in Eucalyptus. *Evolution* 44: 2151–2152.
- Raktoe, B. L., and J. J. Hubert. 1979. *Basic Applied Statistics*. New York: Marcel Dekker.
- Rao, C. R. 1948. The utilization of multiple measurements in problems of biological classification. *Journal of the Royal Statistical Society, Series B* 10: 159–203.
- Reyes Lopez, J. L., and A. Porras Castillo. 1984. Alar biometry in the taxonomy of the species Goniomma hispanicum and G. baeticum. *Insectes Sociaux* 31: 473–475.
- Roger, J. 1863. Die neu aufgeführten Gattungen und Arten meines Formiciden-Verzeichnisses. *Berliner Entomologische Zeitschrift* 7: 131–214.
- Rohlf, F. J., and F. L. Bookstein, eds. 1990. *Proceedings of the Michigan Morphometrics Workshop*. Special Publication Number 2, The University of Michigan Museum of Zoology.

- Ross, K. G., D. J. C. Fletcher, and B. May. 1985. Enzyme polymorphisms in the fire ant, Solenopsis invicta (Hymenoptera: Formicidae). *Biochemical Systematics and Ecology* 13: 29–33.
- Ross, K. G., R. K. Vander Meer, D. J. C. Fletcher, and E. L. Vargo. 1987a. Biochemical phenotypic and genetic studies of two introduced fire ants and their hybrid (Hymenoptera: Formicidae). *Evolution* 41: 280–293.
- Ross, K. G., E. L. Vargo, and D. J. C. Fletcher. 1987b. Comparative biochemical genetics of three fire ant species in North America, with reference to the two social forms of Solenopsis invicta (Hymenoptera: Formicidae). *Evolution* 41: 979–990.
- SAS Institute. 1988. SAS/STAT User's Guide, Release 6.03 Edition. Cary, NC: Sas Institute Inc.
- Say, T. 1836. Descriptions of new species of North American Hymenoptera, and observations on some already described. *Boston Journal of Natural History* 1: 209–305, 361–416.
- Sessions, S. K. 1990. Chromosomes: molecular cytogenetics. In *Molecular Systematics*, ed. D. M. Hillis and C. Moritz, pp. 156–203. Sunderland, MA: Sinauer.
- Smith, D. R. 1979. Superfamily Formicoidea. In *Catalogue of Hymenoptera in America North of Mexico*, Volume 2, ed. K. V. Krombein, P. D. Hurd, Jr., D. R. Smith, and B. D. Burks. Washington, DC: Smithsonian Institution Press.
- Smith, M. R. 1928. An additional annotated list of the ants of Mississippi. *Entomological News* 39: 275–279.
- Smith, M. R. 1930. A list of Florida ants. *Florida Entomologist* 14: 1–6.
- Smith, M. R. 1934. Three new North American ants. *Annals of the Entomological Society of America* 27: 384–387.
- Smith, M. R. 1957. New synonymy of a North American ant, Aphaenogaster macrospina M. R. Smith (Hymenoptera: Formicidae). *Bulletin of the Brooklyn Entomological Society* 52: 113.
- Smith, M. R. 1961. A study of New Guinea ants of the genus Aphaenogaster Mayr (Hymenoptera, Formicidae). *Acta Hymenopterologica* 1: 213–238 + 1 pl.
- Smith, M. R. 1965. House-infesting ants of the eastern United States. Technical Bulletin No. 1326, Agricultural Research Service, United States Department of Agriculture.
- Sneath, P. H. A., and R. R. Sokal. 1973. *Numerical Taxonomy*. San Francisco: W. H. Freeman.
- Spence, J. R. 1990. Introgressive hybridization in Heteroptera: the example of Limnoporus Stål (Gerridae) species in western Canada. *Canadian Journal of Zoology* 68: 1770–1782.

- Steiniger, G. E., and A. B. Mukherjee. Insect chromosome banding: technique for G- and Q-banding pattern in the mosquito Aedes albopictus. *Canadian Journal of Genetics and Cytology* 17: 241–244.
- Stradiotto, J., and M. Stradiotto. 1984. *Discovering Ontario's Parks*. Toronto, Montreal: Grosvenor House Press.
- Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research* 75: 304–306.
- Talbot, M. 1934. Distribution of ant species in the Chicago region with reference to ecological factors and physiological toleration. *Ecology* 15: 416–439.
- Talbot, M. 1951. Populations and hibernating conditions of the ant Aphaenogaster (Attomyrma) rudis Emery. *Annals of the Entomological Society of America* 44: 302–307.
- Talbot, M. 1963. Local distribution and flight activities of four species of ants of the genus Acanthomyops Mayr. *Ecology* 44: 549–557.
- Talbot, M. 1973. Five species of the ant genus Acanthomyops (Hymenoptera: Formicidae) at the Edwin S. George Reserve in southern Michigan. *Great Lakes Entomologist* 6: 19–22.
- Taylor, P. D., and A. Sauer. 1980. The selective advantage of sex-ratio homeostasis. *American Naturalist* 116: 305–310.
- Templeton, A. R. 1989. The meaning of species and speciation: a genetic perspective. In *Speciation and Its Consequences*, ed. D. Otte and J. A. Endler, pp. 3–27. Sunderland, MA: Sinauer.
- Trivers, R. L., and H. Hare. 1976. Haplodiploidy and the evolution of the social insects. *Science* 191: 249–263.
- van der Have, T. M., J. J. Boomsma, and S. B. J. Menken. 1988. Sex-investment ratios and relatedness in the monogynous ant Lasius niger (L.). *Evolution* 42: 160–172.
- Van Pelt, A. F. 1958. The ecology of the ants of the Welaka Reserve, Florida (Hymenoptera: Formicidae). Pt. II. Annotated list. *American Naturalist* 59: 1–57.
- Van Pelt, A. F. 1963. High altitude ants of the Southern Blue Ridge. *American Naturalist* 69: 205–223.
- Varvio-Aho, S., and P. Pamilo. 1980. A new buffer system with wide applicability. *Isozyme Bulletin* 13: 114.
- Ward, P. S. 1980a. Genetic variation and population differentiation in the Rhytidoponera impressa group, a species complex of ponerine ants (Hymenoptera: Formicidae). *Evolution* 34: 1060–1076.

- Ward, P. S. 1980b. A systematic revision of the Rhytidoponera impressa group (Hymenoptera: Formicidae) in Australia and New Guinea. *Australian Journal of Zoology* 28: 475–498.
- Ward, P. S. 1985. The Nearctic species of the genus Pseudomyrmex (Hymenoptera: Formicidae). *Quaestiones Entomologicae* 21: 209–246.
- Ward, P. S. 1989a. Systematic studies on pseudomyrmecine ants: revision of the Pseudomyrmex oculatus and P. subtilissimus species groups, with taxonomic comments on other species. *Quaestiones Entomologicae* 25: 393–468.
- Ward, P. S. 1989b. Genetic and social changes associated with ant speciation. In *The Genetics of Social Evolution*, ed. M. D. Breed and R. E. Page. pp. 123–148. Boulder, CO: Westview Press.
- Wasserman, A. O. 1957. Factors affecting interbreeding in sympatric species of spadefoots (genus Scaphiopus). *Evolution* 11: 320–338.
- Wheeler, W. M. 1915. Some additions to the North American ant fauna. *Bulletin of the American Museum of Natural History* 34: 389–421.
- Wheeler, W. M. 1932. A list of the ants of Florida with the descriptions of new forms. *Journal of the New York Entomological Society* 40: 1–17.
- Wheeler, W. M., and J. F. McClendon. 1903. Dimorphic queens in an American ant (Lasius latipes Walsh). *Biological Bulletin* 4: 149–163.
- White, M. J. D. 1973. *Animal Cytology and Evolution*, 3rd edition. Cambridge: Cambridge University Press.
- Wilson, E. O., and W. L. Brown, Jr. 1953. The subspecies concept and its taxonomic application. *Systematic Zoology* 2: 97–111.
- Wing, M. W. 1968. Taxonomic revision of the nearctic genus Acanthomyops (Hymenoptera: Formicidae). Cornell University Agricultural Experiment Station, Ithaca, NY, Memoir 405, pp. 1–173.
- Woyciechowski, M., and A. Lomnicki. 1987. Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. *Journal of Theoretical Biology* 128: 317–327.

Appendix 1. List of Names for the Genus Aphaenogaster

- aciculata Fr. Smith 1874 [= Messor]
acoreensis Santschi 1933; Azores [desc. as var. of senilis]
acorensis Santschi 1933; Azores [prob. same as acoreensis, but desc. in separate paper]
afra Santschi 1933; N Africa (Algeria) [desc. as var. of depilis, tr. as ssp. of depilis by Cagniant 1968]
alabamensis Wheeler and Wheeler 1934 [= treatae, syn. Creighton 1950]
albisetosa Mayr 1886; SW U.S., Mexico
andrei Mayr 1886 [= Messor]
angulata Viehmeyer 1922; W China [tr. as ssp. of famelica by Chapman and Capco 1951]
anguliceps Viehmeyer 1924; Australia [desc. as var. of longiceps ruginota]
angusta Santschi 1925; Europe (Spain)
annandalei Mukerjee 1930; India [also spelled annandelei in descr.]
anoemica Santschi 1910; N Africa (Morocco) [tr. as var. of sardoa by Emery 1921]
antipodum Fr. Smith 1858 [= longiceps, syn. Wheeler 1916]
aquila Buckley 1867 [nomen nudum in Creighton 1950]
araneoides Emery 1890 [probable syn. of phalangium]
armeniaca Arnoldi 1968; USSR (Armenia) [desc. as subsp. of pallida]
ashmeadi Emery 1895; E U.S.
atlantis Santschi 1929; N Africa (Morocco) [desc. as var. of praedo]
atra Stitz 1938 [= loriai, syn. Smith 1961]
avita Fujiyama 1970 [fossil; Middle Miocene of Japan]
axila Fujiyama 1970 [fossil] + + + +
azteca Emery 1895; Mexico [desc. as var. of fulva aquila]
bakeri Wheeler 1904 [= patruelis] **
balcanica Emery 1898; Turkey, Asia Minor, Greece, "Iles de l'Orient"
barbigula Wheeler 1916; Australia
barcinensis Menozzi 1922 [= gibbosa, syn. Collingwood 1978]
baronii Cagniant 1988; N Africa (Morocco)
beccarii Emery 1887; India, Sumatra
beesoni Donisthorpe 1933; India
belti Forel 1895; Madagascar
berendti Mayr 1868 [fossil; Baltic amber; tr. to Stenamma by Wheeler 1914]
blanci André 1881 [= Goniomma]
borealis Wheeler 1915 [= occidentalis, syn. Creighton 1950]
boulderensis Smith 1941; W U.S.
brevicollis Forel 1899; Panama [prob. syn. of phalangium]
brevicornis Mayr 1886 [= Stenamma]
brevicornis Menozzi 1941; W China [preocc.; desc. as ssp. of smythiesi; in Eidmann; date 1942?]
cabylica Stitz 1917; N Africa (Algeria) [tr. as ssp. of testaceopilosa by Emery 1921]
caeciliae Viehmeyer 1922; W China
calderoni Forel 1914 [= Manica bradleyi, syn. Emery 1921]]
campana Emery 1878; Europe (Italy; Maltese Islands)
canalis Enzmann 1947 [probable syn. of phalangium]
canescens Emery 1895; N Africa (Algeria, Tunisia) [tr. as ssp. of testaceopilosa by Emery 1921]
carbonaria Pergande 1894; Mexico

- cardenai Espadaler 1979; Europe (Spain)
carolinensis Wheeler 1915; E U.S.
cavernicola Donisthorpe 1938; India
ceconii Emery 1894; Crete
chorassanica Arnoldi 1968; USSR (Turkmenia)
clara Santschi 1915; Madagascar [tr. as var. of swammerdami by Emery 1921]
clavata Emery 1877; [= Pheidole in Emery 1921]
cockerelli André 1893; Mexico, SW U.S. [spelled cocquerelli by Emery 1921]
confinis Santschi 1933; N Africa (Morocco) [desc. as var. of praenoda; desc. from males only]
conica Viehmeyer 1913; Europe (Spain) [Collingwood 1978 synonymized "conica Viehmeyer" under iberica; is ionica a misspelling (or intended spelling) of conica?]
continentis Forel 1911; Burma, Indochina [desc. as var. of longipes; = Pheidole?]
corsica Santschi 1933; Europe (Corsica?) [nomen nudum acc. to Baroni Urbani 1971; desc. as var. of spinosa]
crinimera Cole 1953; New Mexico [prob. syn. of texana]
cristata Forel 1902; Himalayas
crocea André 1881; Europe (Spain), N Africa (Algeria)
croceoides Forel 1890; N Africa (Algeria, Tunisia) [tr. as ssp. of crocea by Emery 1921]
curiosa Santschi 1933; N Africa (Morocco) [desc. as var. of gemella]
curta Forel 1891; Madagascar [tr. as var. of swammerdami by Emery 1921]
cypriotes Emery 1908 [= subterraneoides, syn. Emery 1921; check]
dejeani Cagniant 1982; N Africa (Morocco)
depilis Santschi 1911; N Africa (Algeria, Tunisia)
disjuncta Santschi 1933; N Africa (Morocco) [desc. as var. of senilis]
donisthorpei Carpenter 1930 [fossil; Florissant shale]
dromedarius Emery 1900; New Guinea
dulcinea Santschi 1919; Europe (Italy) [tr. as ssp. of pallida by Baroni Urbani 1971; not in Agosti and Collingwood 1987]
ecalcarata Emery 1895 [= tennesseensis, syn. Creighton 1950]
ellipsoida Santschi 1933; N Africa (Morocco) [desc. as var. of praedol]
ensifera Forel 1899; Mexico
epinotalis Wheeler and Mann 1914; Haiti [poss. syn. of relicta]
epirotas Emery 1895; Europe (Greece, Albania, Italy)
espadaleri Cagniant 1984; N Africa (Morocco)
etrusca Baroni Urbani 1968; Europe (Italy) [tr. as ssp. of spinosa by Baroni Urbani 1971]
exasperata Wheeler 1921; China
expolitus Azuma 1950 [nomen nudum acc. to Onoyama 1980, as no description given]
fabulosa Arnoldi 1968; USSR (Turkmenia)
famelica Fr. Smith 1874; Japan, China
faureli Cagniant 1968; N Africa (Algeria) [desc. as ssp. of crocea]
feae Emery 1889; Burma
festae Emery 1915; Europe (Rodhos)
finzii Müller 1921; Europe (Italy)
fiorii Emery 1915; Europe (Sicily, Sardinia) [tr. as ssp. of gibbosa by Baroni Urbani 1971]
flava Emery 1921?; Australia [tr. as var. of longiceps in Emery 1921; possibly first use of this name; figured in Emery 1914; check]
flemingi M. R. Smith 1928; E U.S.
floridana M. R. Smith 1941; E U.S.

- friedericici Forel 1918; Madagascar
frontosa Wheeler 1928; Japan [tr. as ssp. of famelica by Chapman and Capco 1951]
fuentei Medina [= senilis, syn. Collingwood 1978; only ref. to this name found]
fulva Roger 1863; U.S.
funkikoensis Creighton 1950 [= lepida, syn. Brown 1954]
furvescens Wheeler 1915 [= texana, syn. Creighton 1950]
fusca Emery 1900 [= dromedarius, syn. M. R. Smith 1961]
gatesi Wheeler 1927 [= Pheidole]
geeji Wheeler 1921; China
gemella Roger 1862; Europe (Spain incl. Balearic Islands, Portugal)
georgica Arnoldi 1968; USSR
gibbosa Latreille 1798; Europe (France, Italy, Spain, Portugal, Greece, Bulgaria); N Africa (Algeria)
gigantea Collingwood 1962 [prob. Myrmica ritae or M. subritae, acc. to Brown pers. comm.]
gonacantha Emery 1899; Madagascar [gonacanta on fig.; date 1900 in Emery 1921]
gracilinodis Emery 1878 [= Messor rofotestaceus, syn. Emery 1921]
grata Santschi 1933; Europe (Spain) [desc. as var. of senilis]
haarlovi Collingwood 1960; Himalayas?
harnedi Wheeler 1919 [= ashmeadi, syn. Creighton 1950]
hesperia Santschi 1911; Canary Islands
hispanica Andre 1882 [tr. to Goniomma]
holtzi Emery 1898; Asia Minor
homonyma Emery 1921; N Africa (Tunisia), Caucasus [n. n. for subterraneoides Forel, preocc.; tr. as var. of gibbosa]
honduriana Mann 1922; Honduras
huachucana Creighton 1934; SW U.S.
iberica Emery 1908; Europe (Spain, Portugal)
ichnusa Santschi 1925 Europe (Sardinia) [tr. as var. of subterranea by Baroni Urbani 1971]
imbellis Emery 1900; Sumatra [tr. as var. of feae by Chapman and Capco 1951; date 1901?]
inermis Forel 1899 [desc. as var. of araneoides, prob. syn. of phalangium]
inermis Emery 1908; Europe (S Italy, Maltese Islands) [preocc.?; tr. as good sp. by Schembri and Collingwood 1980]
ionia Emery 1915; Europe (Italy, Sicily, Greece, Maltese Islands)
ionica Viehmeyer [= iberica, syn. Collingwood 1978; misspelling of conica?]
isekram Bernard 1977; N Africa (S Algeria)
italica Emery 1916; Europe (Italy) [tr. as var. of gibbosa strioloides by Emery 1921; tr. as good sp. by Baroni Urbani 1971 but not in Agosti and Collingwood 1987]
januschevi Arnoldi 1976; USSR
japonica Forel 1911; Japan [tr. as ssp. of syriaca by Emery 1921, tr. as ssp. of smythiesi by Chapman and Capco 1951]
ionia Emery 1915 [misspelling of ionia by Emery 1921]
juliana Pergande 1894 [= Messor]
kervillei Forel 1910; Syria
kurdica Ruzsky 1905; USSR (Caucasus) [tr. as ssp. of smythiesi by Emery 1921]
laevior Emery 1887; Celebes
laevior Forel 1892; Europe (S Spain) [preocc.; = gibbosa, syn. Collingwood 1978]
laevis Mayr 1862 [= tennesseensis, syn. Forel 1886]
lamellidens Mayr 1886; E U.S.

- laurenti Santschi 1940; N Africa (Algeria) [desc. as var. of pallida]
ledouxii Tohmé 1969; Lebanon
lenis Santschi 1911; N Africa (Algeria, Tunisia) [tr. as var. of crocea by Emery 1921]
lepida Wheeler 1930; Taiwan [n. n. for silvestrii Wheeler 1929, preocc.]
lesbica Forel 1913; Europe (Greece, Yugoslavia, Lesbos)
leveillei Emery 1881; N Africa (Algeria) [tr. as var. of pallida by Emery 1921]
longaeva Scudder 1877 [fossil; genus uncertain acc. to Carpenter 1930]
longiceps Fr. Smith 1858; Australia
longispina Wolf 1914; N Africa (Algeria) [desc. as var. of sardoa; missing from Emery 1921]
loriai Emery 1897; New Guinea
lustrans M. R. Smith 1961; New Guinea
macrospina M. R. Smith 1934 [= flemingi, syn. M. R. Smith 1957]
maculata Theobald 1937 [fossil; French Oligocene]
maculipes Theobald 1937 [fossil; French Oligocene]
manni Wheeler 1917 [= occidentalis, syn. Creighton 1950]
manni Wheeler and Creighton 1934 [= ensifera, syn. Brown 1973]
mariae Forel 1886; E U.S.
marocana Forel 1903; N Africa (Morocco) [tr. as var. of testaceopilosa gemella by Emery 1921, where spelling is maroccana]
masakii Yasumatsu (unpublished?); Japan [probably manuscript name only, on specimens at MCZ]
mauritanica Emery 1891; N Africa (Algeria, Tunisia)
mayri Carpenter 1930 [fossil; Florissant shales]
megommata M. R. Smith 1963; W U.S.
melitensis Emery 1924 [= ionia, syn. Baroni Urbani 1968?]
mersa Wheeler 1914 [fossil; Baltic amber]
mexicana Pergande 1895; Mexico [1896 in Kempf]
miamiana Wheeler 1932; E U.S.
minor Enzmann 1947 [= albisetosa, syn. Brown 1949]
muelleriana Wolf 1914; Europe (Albania, Yugoslavia, Italy)
muschtaidica Ruzsky 1965; Caucasus, Saudi Arabia
mutica Pergande 1895; Mexico
nadigi Santschi 1923; N Africa (Morocco)
nana Wheeler 1932 [unplaced by Creighton 1950; likely syn. of carolinensis]
nicobarensis Forel 1903; Great Nicobar Island [tr. as var. of feae by Chapman and Capco 1951]
nigra Donisthorpe 1938 [= dromedarius, syn. M. R. Smith 1961]
nigripes M. R. Smith 1923 [= lamellidens, syn. Creighton 1950]
nitida Emery 1895; Europe (Italy, Corsica, Sardinia) [tr. as var. of spinosa by Baroni Urbani 1971]
nitidiventris Forel 1912 [desc. as var. of araneoides, prob. syn. of phalangium]
numida Santschi 1933; N Africa (Tunisia) [desc. as var. of depilis]
obsidiana Mayr 1861; Caucasus, Greece, Yugoslavia, elsewhere? **
occidentalis Emery 1895; W U.S.
occidua Santschi 1933; Europe (France, Spain) [desc. as var. of senilis]
ociculata Emery 1899 [= Parapheidole]
oklahomensis Wheeler and Wheeler 1934 [= treatae pluteicornis, syn. Creighton 1950]
oligocenica Wheeler 1914 [fossil; Baltic amber]
opposita Say 1836 [nomen nudum in Creighton 1950]

- osimenseis Teranishi 1940; Japan
ovaceps Emery 1898; Europe (Greece, Italy)
pachei Forel 1906; Nepal [desc. as ssp. of sagei]
pallida Nylander 1848; Mediterranean basin
pannonicus Bachmayer 1960 [fossil; Austrian Pliocene]
patruelis Forel 1885; Mexico, coastal islands of California
pergandei Donisthorpe 1950; SE Turkey [name origin. spelled perganderi but emend. by Donisthorpe 1950; desc. as Ceratopheidole but tr. to Aphaenogaster by Brown 1981, where name is preocc.]
pergandei Mayr 1886 [= Messor]
perplexus M. R. Smith 1961; New Guinea [n. n. for wheeleri Donisthorpe, preocc.]
phalangium Emery 1890; Panama, Costa Rica
phillipi Menozzi 1932 [= lepida, syn. Brown 1954]
phillipsi Wheeler and Mann 1916; Palestine
picea Emery 1895; E North America
picena Baroni Urbani 1970; Italy [1971 on paper] **
pluteicornis Wheeler and Wheeler 1934; U.S. [tr. as ssp. of treatae by Creighton 1950]
poultoni Crawley 1922; Australia [date questionable]
praedo Emery 1908; N Africa (Algeria, Morocco)
praenoda Santschi 1933; N Africa (Morocco)
projectens Donisthorpe 1947; New Guinea [date on journal 1946; desc. from male only]
prudens Forel 1902; Himalayas [tr. as var. of smythiesi by Emery 1921]
pseudoaegyptiaca Emery 1884 [= Messor capensis, syn. Bolton 1982]
punctithorax Cole 1938 [unplaced by Creighton 1950]
pusilla Emery 1895 [unplaced by Creighton 1950]
pythia Forel 1915; New Guinea
quadrispina Emery 1911; New Guinea
relicta Wheeler and Mann 1914; Haiti
rhapsidiiceps Mayr 1877; Turkestan
romana Emery 1895; Europe (Italy) [tr. as var. of spinosa by Baroni Urbani 1971]
rothneyi Forel 1902; Himalayas, central India
rubida Enzmann 1947 [= fulva, syn. Brown 1949]
rudis Emery 1895; U.S.
rufotestacea Donisthorpe 1946; Turkey [desc. as var. of testaceopilosa semipolita]
ruginota Forel 1902 [= longiceps, syn. Wheeler 1916]
rugosoferruginea Forel 1888; Crete [tr. as ssp. of splendida by Emery 1921; desc. has name spelled rugoso-ferruginea]
ruida Wheeler 1928; Japan [tr. as ssp. of famelica by Chapman and Capco 1951]
rupestris Forel 1909; N Africa (Algeria)
sagei Forel 1902; Himalayas, Tibet
saharensis Bernard 1953; N Africa (S Algeria) [desc. from male only]
sangiorgii Emery 1901; Europe (Greece, Cephalonia)
sardoa Mayr 1853; Europe (S Spain, Sardinia, Sicily), N Africa (Algeria, Tunisia)
schmidtii Karawajew 1912; Korea
schmitzi Forel 1910 [tr. as var. of syriaca by Emery 1921]
schurri Forel 1902; India, Burma
semella [ZR 1933; can't find elsewhere; misspelling of gemella?]
semipolita Nylander 1856; Europe (S Italy, Sicily)

- senilis Mayr 1853; Europe (France, Spain, Portugal, Sardinia?)
sicula Emery 1908; Europe (Sicily, Maltese Islands)
silvestrii Menozzi 1929 [= texana, syn. Creighton 1950; likely incorrect synonymy]
silvestrii Wheeler 1929 [preocc.; = lepida Wheeler]
simonellii Emery 1894; Europe (Greece incl. Crete, Turkey, Yugoslavia)
simulans Forel 1915; Simalur (island near Sumatra) [tr. as ssp. of feae by Chapman and Capco 1951]
sinensis Wheeler 1928; China [tr. as ssp. of smythiesi by Chapman and Capco 1951]
smithi Gregg 1949; New Mexico [desc. as ssp. of boulderensis]
smythiesi Forel 1902; Himalayas
sommerfeldti Mayr 1868 [fossil; Baltic amber]
sonorae Pergande 1894 [= cockerelli, syn. Creighton 1950?]
sordida Forel 1892 [= Messor]
spinipes Santschi 1911; Madagascar [tr. as var. of swammerdami by Emery 1921]
spinosa Emery 1878; Europe (Italy, Corsica, Sardinia)
splendida Roger 1859; Europe (Greece, Yugoslavia, Spain, Maltese Islands, Italy, Sicily), Syria, N Africa (Algeria, Tunisia)
splendidoides Forel 1890; N Africa (E Algeria) [tr. as var. of crocea by Emery 1921]
sporadis Santschi 1933; Europe (Greece - Ikaria of the Dodecanese Islands) [desc. as var. of simonellii]
striativentris Forel 1895; Europe (S Spain)
striola Roger 1859 [= gibbosa, syn. Emery 1921?]
strioloides Forel 1890; Europe (S Spain), N Africa (Algeria, Tunisia)
subcostata Viehmeyer 1922; Turkey
subrubra Buckley 1867 [= tennesseensis]
subterranea Latreille 1798; Europe, Crimea, Caucasus, Asia Minor, China
subterraneoides Emery 1881; Europe (Cyprus, Dalmatia, Ionian Islands, France, N Spain)
subterraneoides Forel 1890 [preocc., = homonyma]
subterraneosplendida Emery and Forel 1879 [= sicula]
swammerdami Forel 1886; Madagascar
syria Forel 1910 [= syriaca, syn. Emery 1921?]
syriaca Emery 1908; Syria
takahashii Wheeler 1930; Taiwan
tennesseensis Mayr 1862; E North America
testaceopilosa Lucas 1846; Europe (Spain, S France), N Africa (Algeria, Tunisia), Azores, Canaries, Sardinia, Caucasus
texana Emery 1895; S U.S., Mexico
theryi Santschi 1923; N Africa (Morocco)
tibetana Donisthorpe 1929; Himalayas
tingitana Santschi 1933; N Africa (Morocco) [desc. as var. of testaceopilosa gemella]
tipuna Forel 1913; Taiwan, Japan [tr. as ssp. of rothneyi by Chapman and Capco 1951; excl. from list of Japanese ants by Onoyama 1980]
torossiani Cagniant 1988; N Africa (Morocco)
transcaucasica Karawajew 1926; USSR
treatae Forel 1886; North America
turkestanica Arnoldi 1976; USSR (Turkestan)
uinta Wheeler 1917; W U.S.
uihelyii Szabó 1910; N Africa (Tunisia)

valida Wheeler 1915 [= occidentalis]
vapida Wheeler 1928; Japan
verecunda Wheeler 1928; Japan
vieirai Emery 1908; Europe (Portugal) [tr. as var. of testaceopilosa iberica by Emery 1921]
weigoldi Viehmeyer 1922; China
weulersseae Cagniant 1989; N Africa (Morocco)
wheeleri Mann 1915 [= treatae, syn. Creighton 1950]
wheeleri Donisthorpe 1938 [preocc., = perplexus]
willowsi Wheeler 1933 [= patruelis, syn. Creighton 1950]
wilsoni Cagniant 1988; N Africa (Morocco)

Missing Names

According to Fujiyama (1970), Heer (1849, 1867) described two fossil species from the Miocene Radoboj Beds. I have seen Heer (1867), but can't recognize which described species might be an Aphaenogaster.

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