Insectes soc. 46 (1999) 171–178
0020-1812/99/020171-08 $ 1.50+0.20/0

Insectes Sociaux

Research article

Worker size variation and the evolution of an ant-plant mutualism: Comparative morphometrics of workers of two closely related plant-ants, *Petalomyrmex phylax* and *Aphomomyrmex afer* (Formicinae)

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Received 20 February 1998; revised 18 August 1998; accepted 24 August 1998.

Summary. We compared intranidal variation in worker size in the two closely related plant-ants *Aphomomyrmex afer* and *Petalomyrmex phylax*. Each of these genera is monotypic, and the two appear to be sister species among extant ants. Workers of *A. afer* are larger on average and exhibit much greater intranidal size variation. Workers of *P. phylax* are smaller and much less variable in size. Both species show weak allometry for some pairs of characters. Head shape is also different in workers of the two species. We discuss these differences in relation to the ecology of *A. afer* and *P. phylax*, and propose a scenario for the evolutionary divergence of worker morphology in these two species. Based on comparisons of these two monotypic genera with related ants, we suggest that reduced intranidal variation in worker size is a derived trait in *Petalomyrmex*.

Key words: Worker polymorphism, ant-plant interaction, mutualism, morphometrics.

Introduction

Among the great diversity of ecological lifestyles encountered in ants, representatives of at least 29 genera live in myrmecophytic plants, nesting in specialised structures (domatia) such as swollen stems. Most "plant-ants" are involved in mutualistic associations with their hosts, in which nest sites and food rewards are exchanged for protection from herbivores or competing plants (for a review see Davidson and McKey, 1993a, b). Biological traits involved in the plant-protective behaviour of such ants have been well studied in several species (e.g., Janzen, 1967; Fonseca, 1993; McKey, 1984), but little attention has focused on morphology and morphometry of plant-ants in relation to these mutualisms (Beattie, 1985; McKey, 1988). Selective pressures acting on size and on size variation in these ants include novel components. As in other twig-nesting ants, the dimensions of the plant's domatia may place constraints on size and shape of occupants (Davidson and McKey, 1993a). The size and shape of entrance holes into the domatia, often made in preformed structures of the plant and thus under the plant's partial control, also constrain ant size and shape (e.g., McKey, 1984). The ant inhabitants of some myrmecophytes must navigate among the host plant's dense trichomes (Davidson et al., 1989).

Worker size and its variation are partly a product of selection acting on how the colony's investment in a worker force is allocated. The trade-off between worker size and worker number also includes novel features in ants specialised for life in myrmecophytes. Because ant fitness depends largely on how well they protect their host, worker size, like other traits important in host defence, may be expected to vary between systems in relation to the requirements for plant protection (Gaume et al., 1997). Some plant-ants are very large, such as the ferociously stinging *Tetraponera aethiops* (Pseudomyrmecinae) which protect *Barteria fistulosa* against herbivores as large as *Colobus* monkeys (McKey, 1974) or even elephants (Janzen, 1972). Others are very small, such as the *Pheidole* living in Central American *Piper* (Letourneau, 1983) or South American *Maieta* (Vasconcelos, 1991), and the African *Petalomyrmex phylax* in *Leonardoxa africana* (McKey, 1984). Effectiveness of the

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protection that could be provided by such small ants has sometimes been placed in doubt, but at least three field studies have demonstrated that these small and "timid" ants protect their host plant against its herbivores, which are small insects (Letourneau, 1983; Vasconcelos, 1991; Gaume et al., 1997).

Despite the potential importance of worker size and its variability in the functioning of these mutualisms, worker size range and the degree of polymorphism are aspects usually ignored by biologists working on these associations. Plant-ants may offer opportunities to approach some questions of general importance in worker evolution. Because the host plant (to which foraging activities are restricted, in most cases) represents a simplified environment in comparison to the foraging territories of most ant species, the relationship between worker form and function might be more easily explored in symbiotic plant-ants than in most other ecological categories of ants. This simplified environment might also act to reduce worker size variability, both via the direct effects of lower variability in resource abundance, and via the indirect effects of selection favouring the most adapted phenotype in a homogeneous environment.

We focus here on two related formicines inhabiting ant-plants in rainforest in Cameroon, *Aphomomyrmex afer* and *Petalomyrmex phylax*, which contrast strongly in both mean worker size and intranidal variation in worker size. These two genera are both monotypic (Snelling, 1979), and *A. afer* and *P. phylax* are sister species (Chenuil and McKey, 1996). While *P. phylax* is completely restricted to *Leonardoxa africana* sensu stricto (Leguminosae: Caesalpinioideae), *A. afer* has been collected from two host plants, *L. africana* taxon T3 and *Vitex grandifolia* (Verbenaceae) (Chenuil and McKey, 1996). McKey (1984) described the highly specialised relationship between *P. phylax* and *L. africana*, and protection by this ant has been demonstrated by Gaume et al. (1997). The relationship between *A. afer* and its host plants is less specialised. In contrast to *L. africana* sensu stricto, the host plants of *A. afer* provide few food resources directly to ants, and *A. afer*, in contrast to *P. phylax*, tends homopterans inside the domatia (McKey, 1991; Gaume et al., 1998). Snelling (1979) described the workers of *P. phylax* as monomorphic and those of *A. afer* as polymorphic, but there has been no quantitative study of worker morphometrics in these two species. In this study, we present results describing polymorphism of *A. afer* workers based on analysis of several colonies, compare worker morphometry in *A. afer* and *P. phylax*, and propose hypotheses to account for the divergence of worker size and intranidal variation in worker size since the evolution of these two ant species from their common ancestor.

Materials and methods

Samples of workers of the two species were drawn from sexually mature colonies, in large trees, preserved in alcohol. Colonies of *P. phylax* were collected near Ebodié (2°35′N, 9°50′E), Southern Province, Cameroon, in 1995, 1996 and 1997 (in March all 3 years). Colonies of *A. afer* were collected from three sites in the Southwest Province, Cameroon: two sites in Korup National Park (5°00′N, 8°45′E), Rengo Rock in March 1983 and Iriba Inéné in January and November 1996, and a third site near Nguti (5°18′N, 9°26′E) in November 1989 and March 1995. In the laboratory, workers were removed from alcohol, dissected and dried at room temperature. Head, scape and hind leg of each worker were mounted on double-sided adhesive tape to achieve a standardised orientation and thus ensure adequate precision of measurement. Four measurements were taken on each worker:

1) head length, from the beginning of the clypeus in top view to the end of the head capsule;
2) head width (between the eyes) at the interocular line;
3) scape length (straight-line distance from base to apex of the scape, which is curved);
4) length of the tibia of the hind leg.

All measurements were made using an electronically assisted monocular lens (Nikon Measuroscope 10) under 30× or 50× magnification.

For both species, repeatability of these measurements was determined by performing the same measurements six times on each of a series of individuals. Coefficients of variation of these repeated measurements are 0.49–2.86%, depending on the part measured, for *P. phylax* ($N = 6$ individuals) and 0.17–3.02% for *A. afer* ($N = 9$ individuals). These error values are small compared to variation between individuals in the same colony.

For *A. afer* we measured a total of 2,087 individuals (200–222 from each colony). For *P. phylax* we measured 96 individuals from one colony, supplementing our sample of this species with measurements of a total of 103 workers from 3 other colonies (35, 35, and 33 workers, respectively; L. Amsellem, unpublished data). In all cases our samples were taken haphazardly and form a small proportion of a mature colony.

For interindividual comparisons, coefficients of variation ranged from 7.0–16.3%, depending on the part measured and on the colony, for *A. afer* and from 3.0–4.6% for *P. phylax*. The only body part for which precision of measurement was insufficient to allow analysis of interindividual variation was the scape of *Petalomyrmex*. The scape of both species is curved, making it difficult to ascertain that all measurements were made in the same plane, and in *Petalomyrmex* this part varied little in size between individuals.

In this study, allometry is defined according to Wilson (1971), as the following relation: $\log y = a \cdot \log x + \log b$, where "$x$" and "$y$" denote the dimensions of two body parts. The greater the departure of "a" (the slope of the regression line) from unity, the more pronounced is the differential growth, i.e., the allometry. We used Model I regression to estimate the parameters of the allometric equations. Model I regression is simpler to apply than alternative methods but requires assuming there is no error in the X variable (Harvey and Pagel, 1991). We chose as the X variable in our regressions the body parts for which measures were most repeatable. Departure of slopes from unity was tested using the Proc Reg procedure in SAS (SAS, 1996).

For a sample of 50 workers from a single colony of *Aphomomyrmex*, we determined the relationship between dimensions of the head and dry mass of the entire worker. We considered dry mass to be an estimate of relative amount of resources invested in producing workers of different size. We selected workers to cover a large portion of the intranidal variation in worker size. Each worker was removed from alcohol, dried in an oven at 40°C for four hours, weighed (Sartorius M3P. microbalance, precision 1 µg), put back into alcohol, dissected and prepared for measurement. Storage in alcohol dissolves some body constituents, notably lipids, so that our values for dry mass are underestimates. However, this should not differentially affect workers of different size, so that the values obtained are reliable estimates of relative investment.

Following the same procedures, we also weighed workers and alate females of *Petalomyrmex* and *Aphomomyrmex*, in order to compare the difference between body mass in these two castes for the two species. For each species, all individuals were dried at the same time, but each species was dried separately. Thus, the conditions during drying may have been different, excluding accurate interspecific comparisons of dry weight. We believe that *A. afer* individuals were more thoroughly dried compared to *P. phylax* individuals. We can, however, compare relative mass of individuals of different castes in the same species, since these individuals were dried under exactly the same conditions. For *A. afer*, we weighed a total of 89 alate females and 100 workers from 10 colonies; for *P. phylax* a total of 30 alate females and 81 workers from 8 colonies.

Results

Mean, maximum, minimum, and standard deviation of measurements for the two species are presented in Table 1. Descriptive statistics are given for one colony sample for each species. Results from additional colonies of both species confirm our conclusions.

1) *Polymorphism*

For both species, some pairs of body parts show a very weakly allometric relationship (i.e., *“a”* is slightly but statistically significantly different from one) (Table 2). Workers of *A. afer* exhibit very weak monophasic allometry across a considerable size range within colonies (Fig. 1), but the degree of polymorphism is perhaps less than might be implied by Snelling’s (1979) description, and much less than in most species described as having polymorphic workers (Hölldobler and Wilson, 1990). In *P. phylax*, there is slight but significant allometry for the same pairs of characters as in *A. afer* (Table 2; Fig. 1), but the size range over which this allometry can be expressed is very small, justifying the description of workers as monomorphic (Snelling, 1979).

2) *Comparison of the two species*

Worker size, and the range of worker size within a single colony, are very different in the two species, the magnitude of the difference depending on the body parts considered (Fig. 2). Dimensions of the largest workers of *P. phylax* are only about 20% larger than those for the smallest workers. In contrast, dimensions of the largest workers of *A. afer* are 1.4 to 2 times as large as those for the smallest workers in the same colony. In *P. phylax*, worker size range (maximum value – minimum value) was quite similar among different colonies.

For head length of *P. phylax* the mean size range within colonies (± standard deviation) was 0.078 ± 0.013 mm (N = 4 colonies, a total of 199 workers). Different colonies of *A. afer* differed in mean worker size and in the frequency distribution of size classes (some colonies showed a trend towards bimodality in worker size). For head length of this species, the mean size range within colonies (± standard deviation) was 0.335 ± 0.089 mm (N = 10 colonies, a total of 2,087 workers). Some colonies exhibited less variation in worker size than others, but in all cases, the range of size was greater than within colonies of *P. phylax*.

The two species also differ in the shape of the head, which is relatively broader in *P. phylax* than in *A. afer* (Fig. 1). The

Table 1. Mean, maximum, minimum, and standard deviation of measurements (in mm) for workers from a single colony each of *Aphomomyrmex after* and *Petalomyrmex phylax*. N = 200 workers for all measurements of *A. afer*, N = 96 workers for all measurements of *P. phylax*

Species	Head length			Head width		
	mean	min–max	SD	mean	min–max	SD
Aphomomyrmex after	0.770	0.605–0.992	0.059	0.436	0.348–0.547	0.030
Petalomyrmex phylax	0.501	0.457–0.542	0.015	0.354	0.328–0.376	0.011
	Scape length			Tibia length		
	mean	min–max	SD	mean	min–max	SD
Aphomomyrmex after	0.426	0.333–0.531	0.031	0.559	0.434–0.741	0.044
Petalomyrmex phylax	0.371	0.337–0.414	0.013	0.437	0.393–0.493	0.020

Table 2. Linear regression equations, using log transformation, for pairs of body parts measured for *A. afer (N* = 200 workers) and *P. phylax* (N = 96 workers). Determination coefficients are significant ($P < 0.0001$) in all cases. Slopes “*a*” were tested for departure from 1

y	*x*	*Aphomomyrmex afer*			*Petalomyrmex phylax*		
		Regression	R^2	$P(a=1)$	Regression	R^2	$P(a=1)$
Head width	Head length	$\log y = 0.86 \cdot \log x - 0.26$	0.89	0.0001	$\log y = 0.86 \cdot \log x - 0.21$	0.59	0.0043
Scape length	Head length	$\log y = 0.92 \cdot \log x - 0.27$	0.93	0.0001	$\log y = 0.75 \cdot \log x - 0.26$	0.37	0.015
Tibia length	Head length	$\log y = 0.97 \cdot \log x - 0.14$	0.88	0.21	$\log y = 1.15 \cdot \log x - 0.02$	0.56	0.16
Tibia length	Head width	$\log y = 1.03 \cdot \log x - 0.12$	0.84	0.31	$\log y = 1.10 \cdot \log x - 0.14$	0.56	0.31
Scape length	Head width	$\log y = 0.97 \cdot \log x - 0.02$	0.86	0.28	$\log y = 0.87 \cdot \log x - 0.04$	0.53	0.11
Scape length	Head length	$\log y = 0.86 \cdot \log x - 0.15$	0.86	0.0001	$\log y = 0.54 \cdot \log x - 0.24$	0.44	0.0001

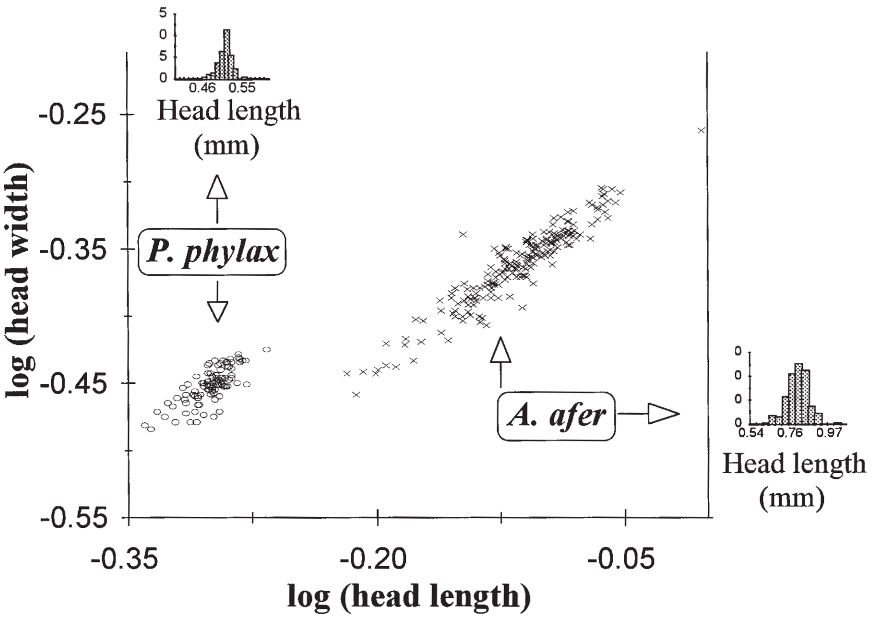

Figure 1. Bivariate plot showing the relationship between head length and head width in workers of a single colony of each of the two species, and size-class distributions of workers of *A. afer* ($N = 200$) and *P. phylax* ($N = 96$)

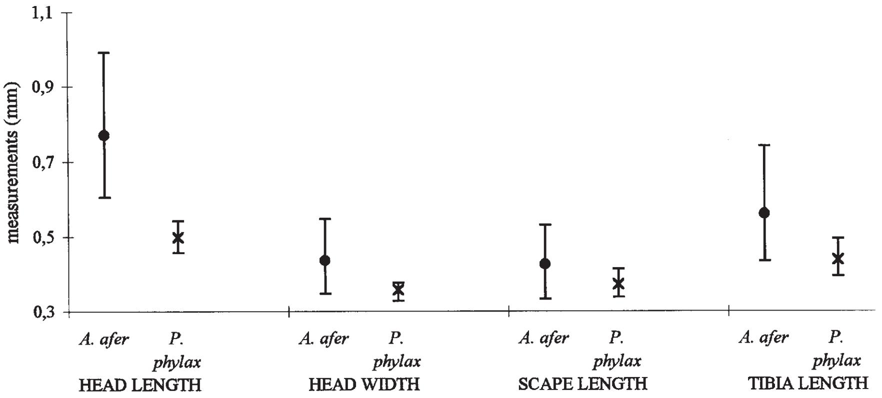

Figure 2. Comparison of maximum, minimum, and mean values (•: *A. afer*; ×: *P. phylax*) for measurements of workers of a single colony of each of the two species. Head length is the best discriminating variable

best discrimination between the two species is given by head length, the sole measure for which values for the two species do not broadly overlap (Table 1).

The large range of linear dimensions among workers of a single *A. afer* colony translates into an even larger range of values for dry mass. For the 50 individually weighed workers of a single colony of this species, dry mass varied from 0.076 mg to 0.273 mg; the mass of the largest worker was 3.6 times that of the smallest. The relationship between dimensions of the head and worker dry mass is described by the following regression equation: Dry mass = $0.489 \cdot$ Head length $\cdot$ Head width $- 0.093$; $R^2 = 0.73$; $N = 50$.

Based on this relationship, we can estimate the range in dry mass per worker in other colonies. In the colony with greatest intranidal variation in worker size, estimated dry mass per worker varied from 0.030 to 0.384 mg, a factor of 12.8.

The ratio (mean female mass/mean worker mass) varies – between colonies – from 19.66 to 38.45 for *P. phylax* and only from 6.74 to 11.68 for *A. afer*. Thus, the smaller size of *Petalomyrmex* is especially characteristic of the worker caste.

Discussion

This study has shown that workers within a colony of *Aphomomyrmex afer* are characterised by a relatively large size range, and exhibit, for some pairs of measurements, a weak monophasic allometry. Workers of *P. phylax* are smaller than those of *A. afer*, and their restricted size range offers little scope for underlying allometry to be expressed. Using Wilson's (1971) criteria for polymorphism (i.e., detectable allometric variation), the distinction between monomorphism and weak polymorphism in such a case may depend more on the investment employed to detect allometry (e.g., accuracy of measurements and number of individuals and measurements compared), than on a biologically meaningful difference. This remark points to the need for consistency in the use of the term "polymorphic": a species with a relatively large size range is not necessarily polymorphic, since worker shape may remain unchanged over the entire size range of its workers. Also, a species which exhibits allometry for some pairs of measurements may be considered monomorphic if the size range is small.

Nevertheless, even in ants with monomorphic workers, size differences may have a functional significance in terms of division of labour (for examples see Hölldobler and Wilson, 1990; Bourke and Franks, 1995). Also, intranidal size variation certainly affects allocation of resources, since large workers are more expensive than smaller ones (Bourke and Franks, 1995). Our results show that the largest workers in an *A. afer* colony contain 3–12 times the dry mass of the smallest worker in the same colony. If metabolic rate does not greatly vary with worker size, this would indicate that the largest workers are 3–12 times more expensive to produce and maintain than the smallest ones. Intranidal variation in worker size (and/or polymorphism) may thus affect both benefits (differential returns from workers of different sizes, based on division of labour) and costs to the colony of its worker force. Because performance of the colony feeds back onto plant fitness, worker size variation may thus be a significant factor in the evolutionary ecology of the mutualism. It is important to note at the outset that the dry mass difference between alate female and worker is much more pronounced for *P. phylax* than for *A. afer*. Size reduction has affected specifically the worker caste of *P. phylax*, and smaller workers cannot be considered simply a pleiotropic consequence of reduced size of queens. An explanation is required specifically for evolutionary reduction in worker size.

Hypotheses to explain why worker size variability might be different in different plant-ants can be grouped into three types:

(1) The size range or degree of polymorphism of each plant-associated ant is advantageous in their respective environments, and may be advantageous to the plant as well. But the "match" results from ecological fitting, e.g., as the outcome of interspecific competition in which these traits conferred an advantage, rather than from evolutionary adaptation of the ant to the host plant. Interspecific competition within guilds of plant-ants probably plays a role in producing the often predictable patterns of ant-plant association (Davidson and McKey, 1993a,b).

(2) The size range or degree of polymorphism is advantageous for the ant, and evolved after the origin of association with the plant, possibly as an adaptation that enhances mutualistic interactions with the plant.

(3) Worker size range or degree of polymorphism confers no particular advantage with respect to the host plant as environment, and simply reflects ancestral characters maintained by phylogenetic niche conservatism or other types of "phylogenetic inertia" (Harvey and Pagel, 1991).

Which of these types of hypotheses might apply to the two ants considered here? We must first know, for the worker characters being considered, which state is primitive and which is derived. *Aphomomyrmex afer* and *Petalomyrmex phylax* are on present evidence sister species among extant ants (Chenuil and McKey, 1996). *Aphomomyrmex* is less specialised (more plesiomorphic) in several behavioural and morphological traits than is *Petalomyrmex* (McKey, 1991), and *Aphomomyrmex* also appears to be closer to the ancestral condition for the traits worker size and intranidal variation in worker size. The ant genera most closely related to these two species on present evidence (Chenuil and McKey, 1996) are all characterised by substantial intranidal variation in worker size. In most *Cladomyrma* spp., mean worker size and the range of variation are similar to those reported here for *Aphomomyrmex* (Wheeler, 1910 [for *Cladomyrma hewitti* (D. Agosti and J. Moog, submitted), as "*Aphomomyrmex hewitti*"]; Agosti, 1991). Allometry in workers has not been studied in this genus, but Agosti (1991) considers the worker caste to be dimorphic. *Gesomyrmex* workers are polymorphic, with workers of *G. kalshoveni* ranging in length from 2.8–6.6 mm (Wheeler, 1929a,b). Wheeler's drawings show that head shape changes over this size range. Snelling and Hunt (1975) describe *Myrmelachista* workers as polymorphic, referring to intranidal size variation. We are aware of no studies of worker allometry in this genus. *Brachymyrmex* workers also often exhibit strong intranidal size variation, and in some species workers are dimorphic, differing in size and shape of the head (for examples, see Santschi, 1923). Available evidence thus strongly suggests that substantial intranidal variation in worker size (and perhaps even polymorphism sensu Wilson [1971]) characterised the common ancestor of *Aphomomyrmex* and *Petalomyrmex*, and that reduced size and monomorphism of *Petalomyrmex* workers are derived traits.

Table 3. Ecological differences between the two species that may be related to differences in worker size and in intranidal size variation

	Aphomomyrmex afer	*Petalomyrmex phylax*
a) Habitat heterogeneity:	• occupies at least two host plant species differing in structure of domatia	• obligately associated with a single host
Worker activities (other than care of brood):	• patrol young leaves • visit foliar nectaries • clean mature leaves • tend at least two homopteran species inside domatia	• patrol young leaves • visit foliar nectaries
b) Constraints on maximum size of workers:	• larger domatia • larger prostoma	• smaller domatia • smaller prostoma

Aphomomyrmex has retained plesiomorphic states of these characters. Hypotheses of type (2) above can thus be excluded for this species, and we must choose between hypotheses of type (1) and (3). The ecological differences between *Aphomomyrmex* and *Petalomyrmex* listed in Table 3 suggest reasons why maintenance of substantial intranidal size variation may be advantageous in *Aphomomyrmex* (points listed under a). With at least two different host plants, the range of environments inhabited by *A. afer* may be more heterogeneous. On its hosts, *A. afer* performs a greater variety of activities, not only patrolling young leaves but also cleaning upper surfaces of mature leaves; and obtaining food not only from visiting foliar nectaries but also from tending at least two species of homopterans. The worker force of this species appears to perform a larger number of different tasks than does the worker force of a *P. phylax* colony. Points listed under b in Table 3 suggest that constraints on maximum size of workers are less severe in *A. afer*, because its host plant produces larger domatia and larger prostomata than does the host of *P. phylax*. The retention of larger workers and of marked intranidal variation in worker size appears at the very least not to be maladaptive in this species. Whether these ancestral traits give *Aphomomyrmex* an advantage in interspecific competition for *L. africana* T3 (hypothesis 1 above) or whether their retention is simply due to some form of "phylogenetic inertia" (hypothesis 3 above) cannot be discerned from available evidence.

Comparison of occupied domatia of the *Leonardoxa* associated with these two ants shows similarities, but also differences in domatia structure and in the ways domatia are modified by ant activities. In both species, pith (of watery, rather than spongy consistency) appears to degenerate spontaneously, probably making it relatively easy to excavate. The principal differences intrinsic to the plant are the diameter of twigs and domatia (broader in *L. africana* T3) and the number of internodes included in a single domatium. In both species, a domatium corresponds to a single unit of growth; interruption of growth of the twig results in lignification of its apex, so that a woody septum separates the domatium from the new domatium formed during subsequent growth. In *L. africana* sensu stricto, a flush of growth almost always produces only a single leaf-bearing internode, and each domatium is one internode long. In *L. africana* T3, in contrast, a flush of growth usually produces 2–3 leaf-bearing internodes, which form a single continuous domatium with continuous pith. Other differences between the two species result from differences in the way ants modify domatia. *Petalomyrmex* chews entrance holes into its host solely at the prostoma (one per domatium). In contrast, *Aphomomyrmex*, in addition to chewing an entrance hole at the prostoma (one per domatium, in the apical internode), also chews entrance holes at other locations along the twig. Entrance holes at these sites are more difficult to excavate, and might require the intervention of larger workers. A second difference is that the internal surfaces of domatia occupied by *Aphomomyrmex* are much less regular than those occupied by *Petalomyrmex*. The internal surfaces of *Petalomyrmex*-occupied domatia appear to correspond to the boundary between pith and wood. The less regular surfaces of *Aphomomyrmex*-occupied domatia suggest that *Aphomomyrmex* workers might enlarge domatia volume by chewing into wood. This activity might again require the intervention of larger workers.

At least part of the irregularity of inner surfaces of *Aphomomyrmex*-occupied domatia may be due, directly or indirectly, to the presence of coccoid homopterans. As in other myrmecophytes, in older twigs in which secondary growth has increased the distance between the internal surface of the domatia and the vascular tissues from which the homopterans extract sap, the homopterans are nested in depressions. The origin of such depressions, found in coccoid-sheltering domatia of many myrmecophytes, is unknown. Some authors believe that they are chewed by ants (Bailey [1922], p. 388; Wheeler [1942], pp. 51–52 and 86–87), while others believe they are caused by growth-inhibiting secretions of coccoids themselves (Schremmer, 1984). We have no data to assess the role of ants in this regard.

An important difference between *Petalomyrmex* and *Aphomomyrmex* is that while the former is specific to a single host plant, the latter occupies two very different host plants. Twigs of *Vitex*, the other host of *Aphomomyrmex*, differ in several respects from those of *Leonardoxa*. First, they bear no apparent specializations for harbouring ants. Twigs are robust (in keeping with the plant's large leaves), but not swollen, and there is no prostoma; workers must enter the domatia through unspecialized woody tissue. Pith is spongy rather than watery in consistence, and does not spontaneously degenerate. Growth is relatively continuous, and pith is also continuous over long sections, with few woody septa. Workers may be able to enlarge domatia by chewing in the dried pith of previously unoccupied twigs. Absence of a prostoma, and possibly secondary enlargement of nesting cavities, suggest that requirements for excavation may be greater in *Vitex*, which may favour larger workers. These differences between the two host plants of *Aphomomyrmex* imply habitat heterogeneity, which could select for a greater size range of workers in this ant than in the specialist *Petalomyrmex.* While our observations suggest that excavation activities are more extensive and difficult for *Aphomomyrmex* than for *Petalomyrmex*, and might thus require larger workers, we do not know if there is a division of labour in these activities with respect to worker size.

In *P. phylax*, worker size and its intranidal variation have diverged from the common-ancestral condition. Hypotheses of type (1) and (3) can thus be excluded. Is there evidence that the evolution of small monomorphic workers enhances ant-plant mutualism in this species (hypothesis 2)? The ecological differences between *A. afer* and *P. phylax* listed in Table 3 suggest reasons why relatively invariable worker size is advantageous in *P. phylax* (points under a), and why smaller workers are favoured in this species (points under b). On their sole obligate host, *Petalomyrmex* workers perform a narrow range of tasks, patrolling only young leaves and obtaining food from foliar nectaries. Absence of association with homopterans is a derived trait in *Petalomyrmex*. Such associations are widespread in related genera, including *Aphomomyrmex* (McKey, 1991; Gaume et al., 1998), *Cla-*

domyrma (Maschwitz et al., 1991), *Myrmelachista* (Davidson and McKey, 1993a), and *Brachymyrmex* (Santschi, 1923). *Gesomyrmex* nest chambers in living plant stems found in Malaysia and Philippines did not contain any homopterans. It is not known whether the ants tend homopterans outside the nest on the plant surface, however, this was never observed (U. Maschwitz, J. Moog and A. Weissflog, pers. comm.). The more restricted range of activities of *P. phylax* workers may favour relatively constant worker size. The smaller domatia and prostoma of its host (C. Brouat, unpubl. data) may restrict maximum worker size in *P. phylax*.

Possessing uniformly small workers may confer an important advantage in *Petalomyrmex* – more of them can be produced from the same amount of resources. *Leonardoxa* is a forest understorey tree, and produces food resources for ants at a lower rate compared to myrmecophytes of light-rich habitats (McKey, 1984). Gaume et al. (1997) argue that partitioning of limited resources into a large number of small workers enables a *P. phylax* colony to control a territory that, compared to many myrmecophytes, is large in relation to the amount of food resources it provides. Small size of these workers is no handicap in plant protection, because the plant's herbivores are themselves very small (Gaume et al., 1997).

Another reason may be suggested to explain why small workers have evolved in *Petalomyrmex*. The host plant of *A. afer*, *L. africana* T3, only begins to produce habitable domatia once the plant has reached substantial size, in general around 1 m height. *A. afer* is usually not the first occupant of these juveniles. Plants usually acquire a colony of *A. afer* above around 5 m height (Gaume and McKey, 1998). At this stage, trees possess several domatia habitable by *A. afer*. In contrast, the host of *Petalomyrmex*, *L. africana* sensu stricto, produces habitable domatia already as a seedling, usually before reaching 30 cm height, and is rapidly colonised by ants. The domatia of these juvenile *L. africana* are especially small. Because nest sites are likely to be limiting, and because the first colony to occupy a plant is likely to enjoy a priority advantage, selection favoured ants capable of using plants with small domatia and low total domatia volume. Small size of workers may have been such a trait, by shortening the time and minimising the space required to produce enough workers to begin foraging and patrolling. According to this hypothesis, high herbivore pressure drove a coevolutionary process in which selection favoured traits of both plants and ants that allowed earlier establishment of the protective mutualism. This hypothesis suggests that the small size of *P. phylax* workers is functionally analogous to the widespread phenomenon of nanitic workers (Hölldobler and Wilson, 1990) in fledgling ant colonies. We suggest the hypothesis that the evolutionary reduction of worker size in *P. phylax* may represent in developmental terms the retention of nanitic workers in mature colonies. A partial test of this hypothesis would be to compare worker size and form in fledgling and mature colonies of *A. afer* and *P. phylax*. Predictions are that (i) worker size varies less between fledgling and mature colonies in *P. phylax* than in *A. afer*, and (ii) size and shape of nanitic workers of *A. afer* are more similar to those of *P. phylax* than are workers of mature colonies of *A. afer*.

Our comparison of worker morphometrics in these two species leaves several open questions. First, to what extent is intranidal variation in size of *Aphomomyrmex* workers due to size differences among overlapping cohorts of workers produced at different seasons? Such among-cohort differences contribute to intranidal variation in worker size in other ant species with weak worker polymorphism and may be adaptive in maintaining worker number in the face of seasonal variation in resource abundance (Rissing, 1987). Secondly, are the selective pressures that currently maintain small worker size in *Petalomyrmex* the same as those that favoured this trait at its origin? Third, what is the significance of other morphological differences between *Aphomomyrmex* and *Petalomyrmex*? The workers of *Petalomyrmex* do not simply represent a truncated segment of the size range of those of *Aphomomyrmex* (Fig. 1). They also differ in shape of the head, palpal segmentation, scape length and location of the ocelli (Snelling, 1979).

This study documents morphometric differences in workers of two closely related arboricolous ants, and argues that they can be explained in terms of divergent adaptation to host plants. One approach to testing this adaptationist hypothesis is to determine whether patterns are repeated in independently evolved plant-ants. While we expect mean size of workers to increase or to decrease in different plant-ants, depending for example on the size of the enemies against which they protect their host (Gaume et al., 1997), we predict that intranidal variation in worker size will decrease in specialist plant-ants, relative to their more opportunistic ancestors. This difference should be most marked when increasing specialisation is accompanied by (1) evolution in the host plant of traits such as the prostoma that reduce the extent of excavation activities; and (2) simplification of the trophic resources exploited by ants, for example, the loss of trophobiotic homopterans and their replacement by a narrow range of resources produced directly by the plant.

Acknowledgements

We thank the Ministry of Research and Higher Education of the Republic of Cameroon for permission to carry out the field component of the study. This study was funded by a grant from the French government (ACCSV 7). J.-C. Auffray (Institut des Sciences de l'Evolution de Montpellier) is thanked for letting us use the measuroscope in his laboratory. We thank L. Amsellem for allowing us to use unpublished data. The manuscript was improved by discussions with C. Brouat about allometry and with M. Gibernau on statistical points, and by comments of J. Moog, U. Maschwitz and an anonymous reviewer. Luc and Marie Moliko are thanked for their hospitality in Ebodié. Alain Ngomi, Gabriel Moky and Ferdinand Namata are thanked for their able assistance in the field. This is publication no. 98-069 from the Institut des Sciences de l'Evolution, Montpellier.

References

Agosti, D., 1991. Revision of the oriental ant genus *Cladomyrma*, with an outline of the higher classification of the Formicinae (Hymenoptera: Formicidae). *Syst. Entomol. 16*: 293–310.

Bailey, I.W., 1922. Notes on Neotropical ant-plants. I. *Cecropia angulata* sp. nov. *Botanical Gazette 74*: 369–391.

Beattie, A.J., 1985. *The Evolutionary Ecology of Ant-plant Mutualisms.* Cambridge University Press, Cambridge. pp. 1–182.

Bourke, A.F.G. and N.R. Franks, 1995. *Social Evolution in Ants.* Princeton University Press, Princeton, New Jersey. 529 pp.

Chenuil, A. and D.B. McKey, 1996. Molecular phylogenetic study of a myrmecophyte symbiosis: did *Leonardoxa*/ant associations diversify via cospeciation? *Mol. Phylogenetics Evol. 6*: 270–286.

Davidson, D.W. and D.B. McKey, 1993a. The evolutionary ecology of symbiotic ant-plant relationships. *J. Hym. Res. 2*: 13–83.

Davidson, D.W. and D.B. McKey, 1993b. Ant-plant symbioses: stalking the Chuyachaqui. *Trends Ecol. Evol. 8*: 326–332.

Davidson, D.W., R.R. Snelling and J.T. Longino, 1989. Competition among ants for myrmecophytes and the significance of plant trichomes. *Biotropica 21:* 64–73.

Fonseca, C.R., 1993. Nesting space limits colony size of the plant-ant *Pseudomyrmex concolor*. *Oikos. 67*: 473–482.

Gaume, L. and D.B. McKey, 1998. Protection against herbivores of the myrmecophyte *Leonardoxa africana* (Baill.) Aubrév. T3 by its principal ant inhabitant *Aphomomyrmex afer* Emery. *C.R. Acad. Sc., Paris, Life Sciences 321*: 593–601.

Gaume, L., D.B. McKey and M.C. Anstett, 1997. Benefits conferred by "timid" ants: active anti-herbivore protection of the rainforest tree *Leonardoxa africana* by the minute ant *Petalomyrmex phylax*. *Oecologia 112*: 209–216.

Gaume L., D.B. McKey and S. Terrin, 1998. Ant-plant-homopteran mutualism: how the third partner affects the interaction between a plant specialist ant and its myrmecophyte host. *Proc. Royal Soc. London, B 265*: 569–575.

Harvey, P.H. and M.D. Pagel, 1991. *The Comparative Method in Evolutionary Biology.* Oxford University Press, Oxford. 239 pp.

Hölldobler, B. and E.O. Wilson, 1990. *The Ants.* The Belknap Press of Harvard University Press, Cambridge, Mass. 732 pp.

Janzen, D.H. 1967. Interaction of the bull's-horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. *Kansas Univ. Science Bull. 47*: 315–558.

Janzen, D.H., 1972. Protection of *Barteria* (Passifloraceae) by *Pachysima* ants (Pseudomyrmecinae) in a Nigerian rain forest. *Ecology 53*: 885–892.

Letourneau, D.K., 1983. Passive aggression: an alternative hypothesis for the *Piper-Pheidole* association. *Oecologia 60*: 122–126.

Maschwitz, U., B. Fiala, J. Moog and L.G. Saw, 1991. Two new myrmecophytic associations from the Malay Peninsula: ants of the genus *Cladomyrma* (Formicidae, Camponotinae) as partners of *Saraca thaipingensis* (Caesalpiniaceae) and *Crypteronia griffithii* (Crypteroniaceae). 1. Colony foundation and acquisition of trophobionts. *Insectes soc. 38*: 27–35.

McKey, D.B., 1974. Ant-plants: selective eating of an unoccupied *Barteria* by a colobus monkey. *Biotropica 6*: 269–270.

McKey, D.B., 1984. Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. *Biotropica 16*: 81–99.

McKey, D.B., 1988. Promising new directions in the study of ant-plant mutualisms. In: *Proceedings of the XIV International Botanical Congress* (W. Greuter and B. Zimmer, Eds.), Koeltz, Königstein/Taunus. pp. 335–355.

McKey, D.B., 1991. Phylogenetic analysis of the evolution of a mutualism: *Leonardoxa* (Caesalpiniaceae) and its associated ants., In: *Ant-plant Interactions* (C.R. Huxley and D.F. Cutler, Eds.), Oxford University Press, Oxford. 310–334 pp.

Santschi, F., 1923. Revue des fourmis du genre *Brachymyrmex* Mayr. *Anales del Museo Nacional de Historia Natural de Buenos Aires 31*: 650–678.

SAS, 1996. *SAS/STAT user's guide*, release 6.12. Cary, NC: SAS Institute.

Schremmer, F., 1984. Untersuchungen und Beobachtungen zur Ökoethologie der Pflanzenameise *Pseudomyrmex triplarinus*, welche die Ameisenbäume der Gattung *Triplaris* bewohnt [Investigations and observations on the behavioral ecology of the plant ant *Pseudomyrmex triplarinus* which inhabits the ant trees of the genus *Triplaris*]. *Zool. Jb. Syst. 111*: 385–410.

Snelling, R.R., 1979. *Aphomomyrmex* and a related new genus of arboreal African ants (Hymenoptera: Formicidae). *Contrib. Sci. Nat. Hist. Museum Los Angeles County 316*: 1–8.

Snelling, R.R. and J.H. Hunt., 1975. The ants of Chile (Hymenoptera: Formicidae). *Rev. Chilena Ent. 9*: 63–129.

Vasconcelos, H.L., 1991. Mutualism between *Maieta guianensis* Aubl., a myrmecophytic melastome, and one of its ant inhabitants: ant protection against insect herbivores. *Oecologia 87*: 295–298.

Wheeler, W.M., 1910. A new species of *Aphomomyrmex* from Borneo. *Psyche 17*: 131–135.

Wheeler, W.M., 1929a. The identity of the ant genera *Gesomyrmex* Mayr and *Dimorphomyrmex* Ernest André. *Psyche 3*: 1–12.

Wheeler, W.M., 1929b. Note on *Gesomyrmex*. *Psyche 36*: 91–92.

Wheeler, W.M., 1942. Studies of the Neotropical ant-plants and their ants. *Bull Mus. Comp. Zoo. 90*: 1–292.

Wilson, E.O., 1971. *The Insect Societies.* Belknap Press of Harvard University Press, Cambridge, Mass. 548 pp.