

# Evolutionary transitions of complex labile traits: Silk weaving and arboreal nesting in *Polyrhachis* ants

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**Abstract** Understanding the maintenance and evolution of complex group behavioural systems has broad significance to our understanding of social evolution, yet we have little insight into how these systems have evolved. Nest-weaving, a rare group behaviour considered a pinnacle of cooperative action in social insects, involves the coordination of workers and larvae by incorporating larval silk into the nest structure. To investigate the evolution of this complex behaviour in the ant genus *Polyrhachis*, we used comparative analysis and an inferred molecular phylogeny based on three mitochondrial genes COI, COII and CytB, and three nuclear genes EF1 a-F2, Wg and Tf. Our results showed that arboreality and nest-weaving are closely associated, but in contrast to the previous hypotheses, represent the ancestral state in the monophyletic

genus. Nest-weaving within the genus, moreover, is remarkably labile. Arboreality and nest-weaving have been lost and partially regained on at least two occasions: two non-weaving subterranean species (sister taxa likely reflecting a single evolutionary event) have reverted to arboreal nesting habits without regaining the use of silk nests, while a third subterranean species has transitioned to nesting in silk nests on the sides of rocks, obtaining silk from spiders and not their own larvae. The loss of larval cocoons, which is correlated with the most complex form of nest-weaving behavior as typified in *Oecophylla*, has occurred independently on at least two occasions within *Polyrhachis*. The repeated loss of nest-weaving behaviour and its partial regaining within the genus provides the first example of a complex group-level trait that did not arise through behavioural progression from simple to complex states. The evolution and loss of complex group-level traits may be more evolutionarily labile than previously appreciated.

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## Introduction

Social insects are well known for the diverse array of complex behaviours and physical structure they are capable of producing and have played a key role in understanding the origin of complex group-level behaviours from the actions of numerous simple individuals following simple rules (Bonabeau et al. 1997). We now have detailed knowledge on the mechanistic

basis of such diverse phenomena as nest selection in ants and honey bees (Franks and Sendova-Franks 1992; Britton et al. 2002; Franks et al. 2002; Pratt et al. 2002; Seeley and Visscher 2004), the spatial organization of brood in ants and honey bees (Camazine 1991; Franks and Sendova-Franks 1992), and the foraging dynamics and organization of mass recruiting (Deneubourg et al. 1983; Beekman et al. 2001; Burd et al. 2002), group-prey retrieving (Robson and Traniello 1998, 2002) and team-forming ants (Anderson and Franks 2003; Sumpter 2006).

Studies investigating the organization of complex group behaviours in social insects have in turn provided insight to broader questions of collective decision making, organization and optimization in other biological and physical systems (Gordon 1996; Bonabeau et al. 2000; Couzin 2009; Reid et al. 2011), leading to significant advances in our ability to find optimal solutions to such diverse problems as traffic flow (Dussutour et al. 2004), communication networks (Dorigo and Stützle 2004) and clustering algorithms for internet searches (Schockaert et al. 2007). Models for the collective behaviour of robots based on social insects (Deneubourg et al. 1991) have even been extended to combine biotic and abiotic elements (cockroaches and robots) into truly cybernetic systems with enhanced problem solving capabilities (Halloy et al. 2007).

Despite the interest in the mechanistic basis of collective behaviours in a diversity of social insect and other target taxa, our understanding of how these systems may operate within an evolutionary context is far less understood (Traniello and Robson 1995). A variety of studies have explored how self-organising systems (a subset of complex system in general) may operate within an environment of natural selection in either general cases (Richardson 2001; Seeley 2002; Johnson and Lam 2010) or within more specific examples such as the evolution of the division-of-labour (Page and Mitchell 1998; Duarte et al. 2011), a prominent feature of social insect biology. Some researchers have developed frameworks within which to interpret the functional roles or benefits of various collective behaviours as a possible method for usefully comparing and interpreting them within an evolutionary framework (e.g. Robson and Traniello 1999; Dornhaus et al. 2012), while others have utilised comparative methodologies to explore a variety of collective systems. Studies of the evolution of social insects including ant systems such as agriculture in attine fungus-growing ants (Schultz and Brady 2008), worker morphology in *Eciton* army ants (Powell and Franks 2006) and foraging behaviour in *Dorylus* swarm-raid-ers (Kronauer et al. 2007) can all be considered studies of the evolution of complex systems. In general, they support the view that ‘simple’ systems precede the evolution of more complex ones and highlight the role comparative studies may play in resolving general patterns of the evolution of complex systems.

The evolution of nest-weaving behaviour in arboreal ants has been considered a pinnacle of cooperative behaviour (Hölldobler and Wilson 1977; Hölldobler and Wilson 1983), yet we have a limited understanding of the individual behaviours involved, the link between individual and group behavior and the socioecological factors that may have been associated with its evolution within ants (Crozier et al. 2009). In its highest grade in the arboreal ant genus *Oecophylla*, workers bring larvae to the work site, which allocate their entire silk supply to bind together the edges of the leaves or other nest substrate and do not produce larval cocoons. The expression of this behaviour at the individual level varies from the lowest grade in arboreal *Dendromyrmex* in which larvae produce silk without any interaction from workers and continue to produce larval cocoons, to an intermediate grade in arboreal *Camponotus senex* and *Polyrhachis ?doddi* (re-described as *Polyrhachis robsoni* (Kohout 2006)) where workers hold larvae at the work site with less ritualized behaviours than those found in *Oecophylla*, but whose larvae may no longer produce cocoons (Hölldobler and Wilson 1977; Hölldobler and Wilson 1983). Only in *Oecophylla* do we have some knowledge of the link between simple individual and complex group behavior, via studies examining the collective dynamics of chain formation (Lioni et al. 2001; Lioni and Deneubourg 2004) and leaf rolling (Bochynek and Robson 2014). When compared between genera, these stages are considered to represent possible steps in the evolutionary trajectory of nest-weaving behaviour, from simple to complex (Hölldobler and Wilson 1990), but the ubiquity of arboreal nesting habits in all of these examples obscures detecting possible socioecological determinants of nest-weaving. Phylogenetic tests based on molecular sequence data suggest that nest-weaving has evolved independently in each of the four genera in which it occurs (Johnson et al. 2003). *Melissotarsus emeryi* is unique among ants in its possession of hypostomal glands in workers that are used as sources of nest silk in their arboreal nests (Hölldobler et al. 2014).

Recent work involving principally taxonomic and ecological studies indicates that nesting habits and ecology in the taxonomically extensive ant genus *Polyrhachis* are almost as diverse as that of all ants in general (reviewed in Robson and Kohout (2007)), offering the unique potential to explore the evolution of nest weaving within a single genus. Nesting location within *Polyrhachis* species for example can vary from intertidal and subterranean to arboreal, the presence of silk nests and/or larval cocoons is highly variable and disjunct, and even the source of silk within nests can vary from their own larvae to spiders silk. The two studies that have examined the evolution of nesting habits with *Polyrhachis* have produced conflicting hypotheses. Hung (1967), using morphological data, proposed that arboreal nest-weaving and cocoon loss evolved from a subterranean non-weaving and cocoon producing ancestor. Johnson et al. (2003), using molecular

data that included a total of seven species from three subgenera, proposed a monophyletic status for the genus with nest-weaving as the ancestral state.

In this study, we take advantage of the significant behavioural, ecological and taxonomic diversity within the ant genus *Polyrhachis*, to develop phylogenetic hypotheses for the evolutionary relationships between all 13 *Polyrhachis* subgenera (Dorow 1995; Kohout 2010) and use these hypotheses to examine the evolution of complex group-level behaviour, nest-weaving. Do complex systems always arise through a series of graded steps of increasing complexity or are alternative pathways possible?

## Materials and methods

### Taxon sampling

Thirty-seven specimens of ants from the genus *Polyrhachis* (Insecta: *Hymenoptera*: *Formicidae*: *Formicinae*), from all 13 currently recognized subgenera, were included in our analyses. In addition, five outgroup taxa were included from the closely related genera *Camponotus* and *Oecophylla*. Voucher specimens have been deposited in the collections of S.K.A. Robson at James Cook University, Townsville, Australia and in many cases duplicates have also been deposited in the collections of the Queensland Museum, Brisbane, Australia.

### DNA isolation, polymerase chain reaction (PCR) amplification and sequencing

Field collections were made in 95 % EtOH and kept in the laboratory until the time of DNA extraction. Total genomic DNA was isolated for one individual worker using the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocols.

We amplified and sequenced DNA from six fragments using specific primers for each gene region following the protocols of Beckenbach et al. (2005), Ceccarelli and Crozier (2007), Degnan et al. (2004) and Schlüns (2011): cytochrome oxidase I (COI) protein encoding mitochondrial marker, cytochrome oxidase II (COII) protein encoding mitochondrial marker, cytochrome b (CytB) protein encoding mitochondrial marker, elongation factor 1a F2 (EF1a-F2) protein encoding nuclear marker, wingless (Wg) protein encoding nuclear marker and transferrin (Tf) protein encoding nuclear marker for a total of almost 5000 base pairs (bp) of aligned sequence. All sequencing was done using dye terminator cycle sequencing following the protocol specified by the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Revision B, August 1995, Perkin-Elmer, Norwalk, CT). Primers used for amplification served as sequencing primers. All samples were sequenced in both directions.

### Sequence alignment

After sequences were collected, they were analysed and initially aligned using the computer programs Sequencing Analysis 3.7 (ABI Prism 2001) and Geneious v5.6 (Drummond et al. 2012), respectively. Inferred amino acid sequences were used for all genes, allowing for comparatively uncomplicated alignment using Mesquite v2.75 (Maddison and Maddison 2011).

### Phylogenetic analysis

To infer relationships among the species of *Polyrhachis*, several model based phylogenetic analyses were performed on the CIPRES Science Gateway (Miller et al. 2010) using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). In order to evaluate the fit of the data, analyses were conducted using the complete concatenated data set (single), partitioned by individual genes (6 partitions) and partitioned by site-specific models for each codon of each gene (18 partitions). Modeltest 3.06 (Posada and Crandall 2001) was used to determine the most appropriate nucleotide substitution model for each dataset: Single=GTR+G+I; 6 partitions=COI: GTR+G+I, COII: GTR+G+I, CytB: GTR+G+I, EF1a-F2: JC, Wg: HKY+G+I, Tf: GTR+G; and for the 18 partitions: COI position1: GTR+G+I, COI position2: GTR+G+I, COI position3: F81+G+I, COII position1: GTR+G+I, COII position2: GTR+G+I, COII position3: F81+G+I, CytB position1: GTR+G+I, CytB position2: GTR+G+I, CytB position3: F81+G+I, EF1a-F2 position1: JC, EF1a-F2 position2: JC, EF1a-F2 position3: K80+G, Wg position1: HKY+I, Wg position2: GTR+G, Wg position3: HKY, Tf position1: HKY+G+I, Tf position2: HKY+G+I, Tf position3: GTR+G.

Bayesian inference analyses were performed using MrBayes, with model parameters being estimated during the run, and using the default value of four Markov chains. A 'temperature' parameter of 0.2 was implemented to produce incremental heating of each chain. The Markov chain Monte Carlo (MCMC) length was 25,000,000 generations, with the chain sampled every 1000 generations. Bayesian posterior probabilities (BPP) were estimated as the proportion of trees sampled after 10 % burn-in that contained each of the observed bipartitions (Rannala and Yang 1996; Larget and Simon 1999). Again, three analyses were performed: (1) a single model of sequence evolution was assumed to underlie all concatenated gene regions (single), (2) one that allowed each gene region to have a separate model of molecular evolution with parameters unlinked (6 partitions), and (3) one that allowed each codon in each gene to have a separate model of molecular evolution (18 partitions). For parameters across partitions, we unlinked the substitution rates, character state frequencies, gamma shape parameter alpha (G) and invariable

sites (I). All other parameters (i.e., priors) were left at their default values. Independence of runs was insured by only accepting analyses where the average standard deviation of split frequencies was below 0.01.

#### Ancestral state reconstruction

To infer ancestral states for behavioural and nesting preference characters of interest, we implemented ancestral state reconstructions in SIMMAP v1.5.2 (Bollback 2006) and Mesquite v2.75 (Maddison and Maddison 2011). For analyses in SIMMAP, for two-state characters, a beta distribution prior was used for the bias parameter and, for multi-state characters, an empirical prior was used. For all cases, the gamma distribution prior of the rate parameter was assigned  $k=90$ . All other parameters were left in default settings. In Mesquite, we implemented the Mk1 likelihood reconstruction method (with default settings), which maximize the probability the observed states would evolve under a stochastic model of evolution (Schluter et al. 1997; Pagel 1999). In both analyses, we inferred ancestral character states for nesting preference (arboreal versus ground nesting) and silk weaving (larval silk used in nesting material). Taxa were assigned to these categories following Robson and Kohout (2007) or from information from our personal collection records. We defined arboreal as any species nesting in hollow twigs in trees or in nests on leaves attached to living trees. Ground nesting was defined as any species that nests in the soil, in rotten logs on the ground or in or on stones and rock outcrops. To investigate the evolution of nest construction, we assigned species to one of the following categories: silk weaving nests, no silk nest or carton nests (made of small pieces of plant material and other detritus). We also looked at the use of spider silk and whether larval cocoons are present. For both methods, we inferred ancestral states across the distribution of posterior trees from our 18 partitions Bayesian analysis (25,001 trees). Although *Polyrhachis* is very rich species with currently 694 described species (Bolton 2014), the remarkably consistent nesting habits within each of the 13 subgenera, the inclusion of species from all subgenera and the inclusion of species that cover the known diversity of nesting habits (Robson and Kohout 2007) should ensure a robust examination of the evolution of nesting habits within the genus.

#### Character correlation analyses

To test for correlation between character states for nesting location and silk weaving, we implemented Pagel's (1994) test of dependence of character evolution as implemented in Mesquite v2.75 (Maddison and Maddison 2011). This method analyses the relationship between two discrete characters across a phylogeny taking into account branch lengths, develops estimates of rates of change for the characters, and tests

for correlated evolution without relying on ancestral state reconstruction. To discriminate whether a four-parameter or eight-parameter model (one allowing only independence of the evolution of the characters and the other allowing for correlated evolution) is a better fit to the data a likelihood ratio test statistic is used. Since missing data is not permitted for character states, we implemented two analyses: (1) excluded the two taxa (*Polyrhachis lamellidens* and *Polyrhachis striata*) for which one or both characters are unknown and (2) coded these taxa for the least likely coevolved character states (i.e., arboreal+no silk nest and/or ground nesting+silk weaving nest). To estimate statistical significance, we implemented 1000 Monte Carlo simulations in Mesquite under the null hypothesis to calculate  $P$  values. We also used the Huelsenbeck et al. method (Huelsenbeck et al. 2003) for measuring character and state associations as implemented in SIMMAP v1.5.2 (Bollback 2006). In this analysis, the null hypothesis is that characters evolve independently and that associations are the result of chance rather than correlated evolution. For both analyses, we used the 25,001 posterior distributions of trees to test for correlation of traits.

## Results

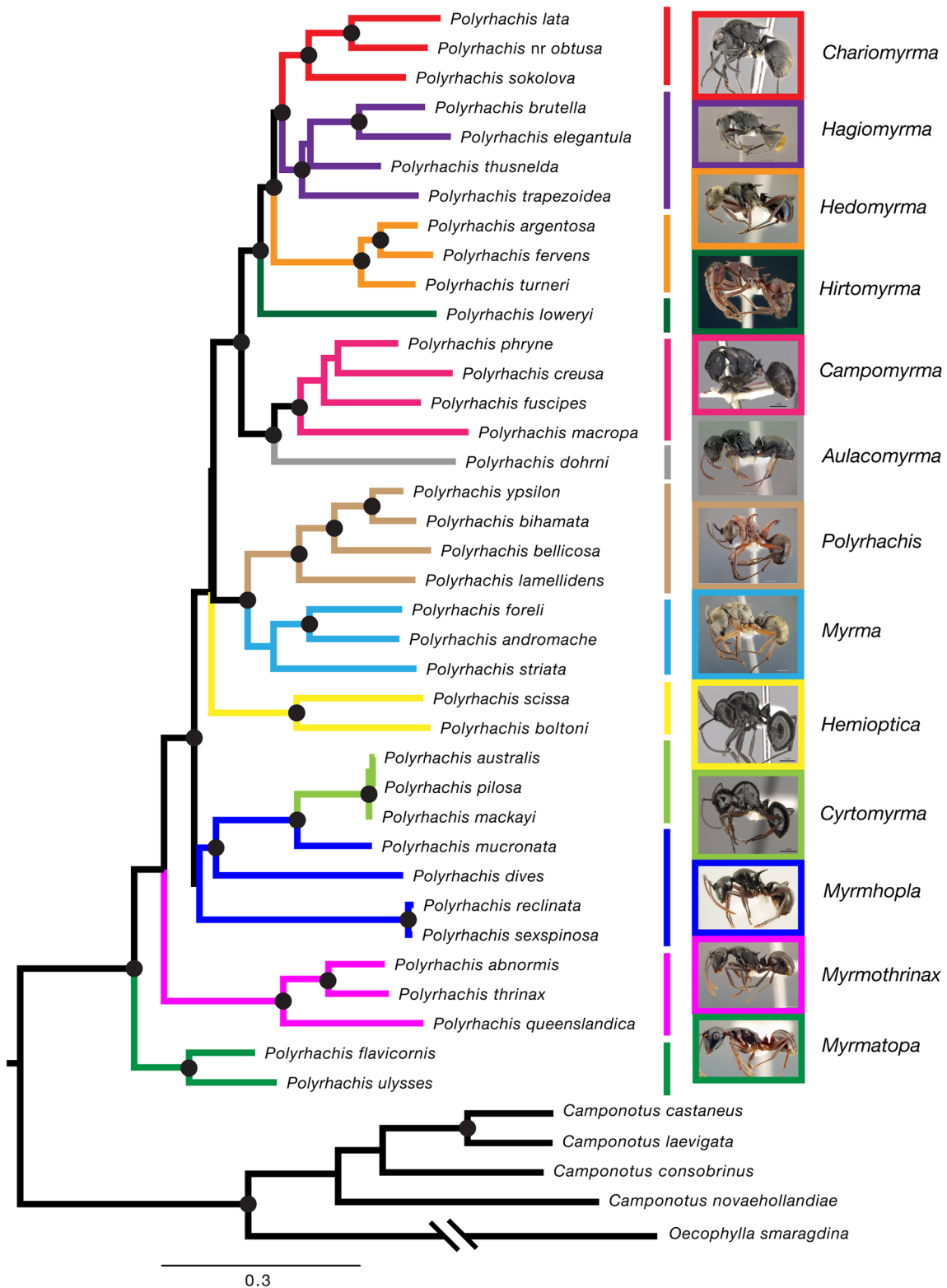
#### Simple sequence statistics

This study produced a final aligned 4976 bp fragment of the following six genes regions: a fragment spanning the mitochondrial cytochrome oxidase I (COI) (750 bp) gene, a fragment spanning the mitochondrial cytochrome oxidase II (COII) (579 bp) gene, a fragment spanning the mitochondrial cytochrome b (CytB) (747 bp) gene, a fragment of the nuclear protein-encoding gene elongation factor 1a F2 (EF1a-F2) (506 bp), a fragment of the nuclear protein-encoding gene wingless (Wg) (354 bp) and a fragment of the nuclear protein-encoding gene transferrin (Tf) (2040 bp). Sequences have been deposited in GenBank (Supplement 1).

#### Phylogenetic analyses

All partitioning schemes resulted in nearly identical topologies with only the few weakly supported clades in conflict (represented on Fig. 1 by a lack of a circle on the nodes). The Bayesian inference topology for the 18 partitions analysis is presented in Fig. 1 with Bayesian posterior probability (BPP) support values  $\geq 0.95$ . All Bayesian inference tree topologies show strong support (1.0 B.P. for the monophyly of the genus *Polyrhachis*). In addition, all subgenera received strong support for their monophyly except *Myrma*, which group together topologically but only had 0.80 B.P. and *Myrmhopla*, which





**Fig. 1** Phylogram of *Polyrhachis* as inferred through Bayesian analysis for the 18 partitions dataset. Subgenera of *Polyrhachis* are noted by colored clades, bars and representative photos of all 13 subgenera (photographs by R. J. Kohout and Antweb.org). Branch lengths are

proportional to substitution/site as indicated by the bottom legend inset. Bayesian posterior probability (BPP) clade support  $\geq 0.95$  is denoted on branches as black circles

we recover as paraphyletic with the *Cyrtomyrma* subgenus nesting within the subgenus as currently defined.

### Ancestral state reconstruction

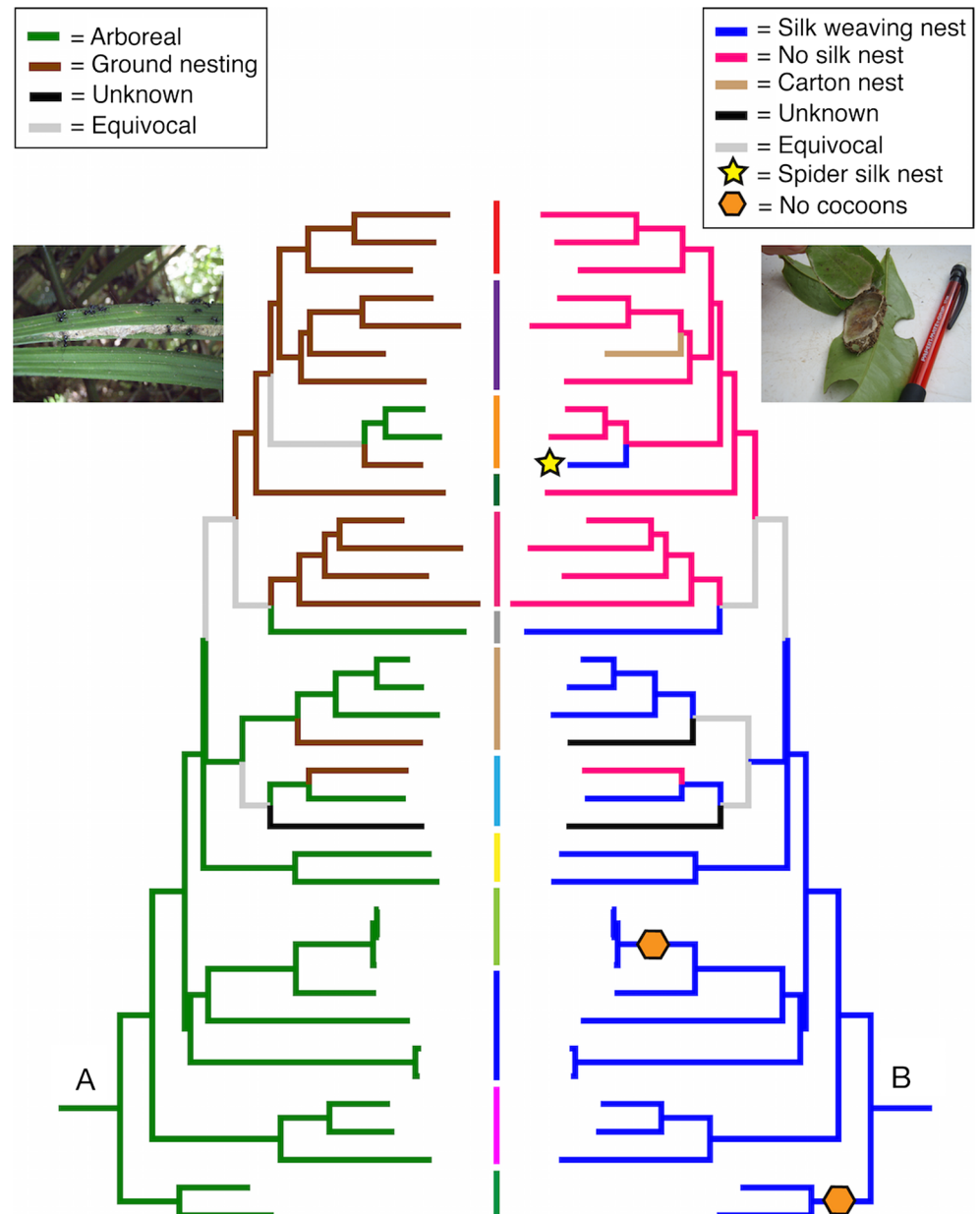
We investigated the nesting preference (arboreal vs. ground nesting) and the evolution of larval silk weaving for nest construction. The results of these analyses are presented in Fig. 2. The results from both the SIMMAP and Mesquite ancestral state reconstructions for both characters highly support the states presented in Fig. 2. All clades received high support (posterior probabilities >0.95) for the reconstructed characters presented in Fig. 2 in both the SIMMAP and Mesquite analyses with few exceptions including the four

subgenera clades within *Polyrhachis*, which had lower than 0.95 BPP support in Fig. 1. Collapsing these clades does not change the overall conclusions of these analyses or directionality of trait evolution. The ancestral state for *Polyrhachis* is strongly inferred to be silk weaving and arboreal nesting (posterior probabilities in both analyses for both traits 0.99), with secondary loss of these characters.

### Character correlation analyses

Tests of correlated characters in Mesquite using Pagel's correlation analysis method (Pagel 1994) strongly supported the correlation between nesting preference and larval silk weaving in both analyses: (1) excluding the two taxa for which one or

**Fig. 2** Ancestral state reconstruction of characters mapped on the phylogram of *Polyrhachis* (same as in Fig. 1; outgroups removed for presentation, but included in all analyses). **a** Nesting location preference. Arboreal nesting denoted in *green* and ground nesting denoted in *brown*. **b** Silk nest weaving behaviour. Silk weaving of nest denoted in *blue* and no silk in nest denoted in *pink*. All silk weaving done using larval silk except for the one taxon denoted by a *yellow star*. Clades that do not have cocoons (naked pupae) are denoted by an *orange hexagon*. For both phylograms presented unknown states are denoted in *black* and clades where the ancestral state reconstruction inferred an equivocal state are denoted in *gray*. Subgenera are denoted by colored bars as in Fig. 1. All clades received high support for the reconstructed characters presented in the figure with the exception of the four subgenera clades within *Polyrhachis*, which had lower than 0.95 BPP support in Fig. 1. Photograph insets demonstrate nests of *Polyrhachis* between leaves with larval silk weaving (photographs by C. S. Moreau)



both characters are unknown (likelihood difference=11.0611;  $P=0.00$ ) and (2) coding these two taxa for the least possible coevolved character states (likelihood difference=9.51860;  $P=0.00$ ). For the analysis of morphological character and state associations implemented in SIMMAP, again silk weaving and arboreal nesting were strongly positively associated with each other (observed values,  $M=0.177516$ ,  $D=0.543009$ ;  $P=0.000399$ ). The results of all correlation analyses were concordant. In every case, silk weaving and arboreal nesting and no silk use and ground nesting were positively associated with each other.

## Discussion

In this study, we provide a well-resolved phylogenetic hypothesis for the highly diverse ant genus *Polyrhachis*, based on 37 species from all 13 subgenera. As a prelude to the discussion of the implications of these results for the evolution of complex systems, we provide a brief discussion of the taxonomic and phylogenetic implications, as congruence with earlier taxonomic studies provides additional confirmation of the validity of the proposed phylogenetic hypotheses and the conclusions drawn from it.

### Taxonomic and phylogenetic implications

The proposed phylogenetic relationships provide a significant support for the monophyly of the genus proposed by Johnson et al. (2003) and the current taxonomic classification of the genus into 13 subgenera based on morphological characters, including the recent elevation of the *Polyrhachis viehmeyeri* group of the subgenus *Myrmhopla* to their own subgenus, *Hirtomyrma* (Kohout 2010). Ten of the 13 *Polyrhachis* subgenera represent monophyletic groups (*Campomyrma*, *Chariomyrma*, *Cyrtomyrma*, *Hagiomyrma*, *Hedomyrma*, *Hemioptica*, *Polyrhachis*, *Myrma*, *Myrmatopa* and *Myrmothrinax*, Fig. 1). Two of the remaining subgenera (*Aulacomyrma* and *Hirtomyrma*) could not be explicitly tested for monophyly as they are only represented in this analysis by a single species in each case, but they represent sister taxa to well-defined clades. The subgenus *Myrmhopla* has long been considered a ‘waste basket’ subgenus for those *Polyrhachis* spp. of uncertain placement (Dorow 1995), a conclusion supported by its lack of monophyly with the subgenus *Cyrtomyrma* nested within the currently defined *Myrmhopla* (Fig. 1). The similarity in morphology and nesting habits of the *Cyrtomyrma* to their sister taxa as indicated in the phylogeny (*Polyrhachis* (*Myrmhopla*) *mucronata*) suggests that the *Cyrtomyrma* may well be a derived *Myrmhopla* clade, but with only three of the 16 *Myrmhopla* species groups (Dorow

1995) represented in this phylogeny it is likely that the subgenus may be even more poorly defined.

The proposed phylogeny (Fig. 1) provides strong support for the monophyly of the genus and the almost uniform presence of monophyletic groups matching current taxonomic groups, presents a fairly straightforward hypothesis for the evolutionary relationships between the 13 subgenera, the biogeographic history of the genus and the evolution of a variety of morphological parameters. The monophyletic terminal grouping of *Chariomyrma*, *Hagiomyrma* and *Hedomyrma*, for example, all share a distinctive box-shaped thorax and are restricted almost entirely to the Australasian region (Dorow 1995; Kohout 2013). The strong biogeographic signature found within the *Polyrhachis* phylogeny reflects patterns being revealed more frequently in a variety of ant groups with the advent of more extensive phylogenetic analyses (Brady 2003; Moreau 2008; Ward et al. 2010; Moreau and Bell 2013).

### Implications for the evolution of complex systems

The derivation of a well-supported phylogeny for the genus *Polyrhachis* in concert with details of the highly diverse nesting habits provides a framework for the comparative analysis of the complex behavioural trait ‘nest-weaving’ and the ecological parameters associated with its evolution.

The phylogenetic conclusions presented here (Figs. 1 and 2) do not support the view that within *Polyrhachis* complex group behaviours such as nest weaving evolved through a series of steps from simple to complex. In contrast to the previous hypotheses involving subterranean ancestors evolving the more complex nest-weaving capabilities as they made the transition to arboreal nesting habits (Hung 1967), but in agreement with the ancestral state predictions of Johnson et al. (2003), we find that the production of arboreal silk nests is the ancestral state for *Polyrhachis*, with at least two transitions to subterranean nesting and the loss of silk weaving as species become more derived. The evolutionary pathways found in *Polyrhachis* are also in contrast with those described from other social insects. Fungus growing within the Attine ants is considered to represent an evolutionary transition through five grades of increasing complexity, ranging from the ‘lower agriculture’ within *Mycocepurus*, *Myrmicocrypta* and *Apterostigma*, involving a wide range of fungal species, through to the ‘higher domestic agriculture’ of *Trachymyrmex* to ultimately the ‘leaf-cutter agriculture’ in the ecologically dominant *Acromyrmex* and *Atta* (Schultz and Brady 2008), involving obligate symbiotic relationships with a single species of highly derived fungi (Poulsen and Boomsma 2005).

The phylogenetic conclusions also reveal a surprising level of flexibility and reversal in the character states of nesting habit and nest-weaving status. The *Hedomyrma* subgenus, for example, is nested within a larger clade of subterranean

nesters that has already lost both arboreality and nest-weaving (Figs. 1 and 2). Yet, two *Hedomyrma* species (*Polyrhachis argentosa* and *Polyrhachis fervens*) have reverted to arboreal nesting, albeit in a slightly different manner than the typical nest-weaving *Polyrhachis*. Rather than construct silk nests on the trunks of trees or the between leaves, they now form nests within the hollow internodes of bamboo, with the absence of any larval silk (Robson and Kohout 2005). The third *Hedomyrma* species included in this analysis, *Polyrhachis turneri*, displays yet another pattern of reversal. This species has adopted lithophilic nesting habits (it nests on the sides of rocks) and although it has reverted to the construction of silk nests, the silk itself is now obtained from a different source. Rather than using larval silk as is typical for the genus, workers steal and use spider silk to construct their nests, with larvae retaining all silk for their own (cocoon) needs (Robson and Kohout 2005). In this case, the character state ‘nest weaving’ has re-evolved, but the mechanism and behaviours underlying it are different. Only workers are involved in silk nest construction in *P. turneri*. The relationship between the retention or loss of larval cocoons and the association with nesting habits also appears to be flexible within the genus. Larval cocoons, the ancestral state for the genus, have been lost in two arboreal nest-weaving species but retained in the remaining majority of arboreal nest-weaving *Polyrhachis*.

Other examples consistent with character reversal of complex group behaviours exist within the social insects. Foraging habits within the army ant genus *Dorylus* show the typical evolutionary transition from simple to more complex group behaviours, with ancestral subterranean foraging transitioning to surface foraging and then leaf-litter foraging. Yet, within the surface foraging clade, there is a secondary reversal to subterranean foraging habits (Kronauer et al. 2007).

The combination of these results with other studies of the phylogenetic history of ants provides a more comprehensive overview of the ecological and life history traits associated with the evolution of ants. We now know, for example, that the transition in nesting habits from arboreal to subterranean and in some case back to arboreal in the genus *Polyrhachis* occurs within the context of a general transition from subterranean to leaf litter and arboreal nesting in ants as whole, when examined at the subfamily and generic level (Lucky et al. 2012).

The original interpretation of Holldobler and Wilson (1983), that the individual behaviors associated with nest weaving in the four Formicine genera *Dendromyrmex*, *Camponotus*, *Polyrhachis* and *Oecophylla* potentially represent stages in the evolution of this behavior within ants, may now need to be revised. Subsequent findings that nest weaving has evolved independently within these four genera (Johnson et al. 2003), coupled with the highly variable relationships between the elements of nest-weaving found in *Polyrhachis* in this study (nesting location, nest material, cocoon presence or absence) suggest much more labile evolutionary patterns and

the decoupling of at least some of the proposed links between these elements. The loss of larval cocoons and the allocation of larval silk to colony rather than individual need, considered to be correlated with the more derived examples of nest weaving (Holldobler and Wilson 1983), appear to be decoupled from nesting habits in *Polyrhachis* where cocoon loss has occurred on at least two occasions in larval silk nest-weaving species (Fig. 1). Similarly, the loss of silk nest weaving within *Polyrhachis* as species transition from arboreal to terrestrial nesting habits, with subsequent re-evolution through the acquisition of spider rather than larva silk as species leave subterranean nesting habits and move back to lithophilic habits, suggests a strong yet flexible link between the behavioural expression of nest weaving and nesting habits. It is unfortunate that despite information on the general nesting habits of over 190 *Polyrhachis* species, details on key social parameters that might further inform our understanding of the evolution of complex group behaviours such as nest weaving within the genus (colony size, queen number and social structure) remain extremely limited (Robson and Kohout 2007). Studies of the behavioural interactions between workers and larvae within individual *Polyrhachis* species would be useful to test Holldobler and Wilson’s (1983) prediction that the behavioural interactions between workers and silk producing larvae become more stereotyped as the contribution of larval silk to nest structure becomes more significant.

The phylogenetic results presented here extend our understanding of the evolution of complex systems, by highlighting an unexpected level of flexibility and reversion in the transition from simple to complex behavioural traits. Complex systems such as nest-weaving behaviour can be readily lost and re-evolved within the evolutionary history of a single genus, highlighting the value of including comparative methodologies in a growing body of approaches to understand how complex systems evolve. Duarte et al. (2011), for example, have suggested examining self-organizing (complex) systems with approaches that emphasize broad-scale colony attributes such as optimal caste ratios and the division of labor between workers. Others have examined the individual behavioural mechanisms underlying such discrete tasks across different species, such as the construction of queen cells in termites and the regulation of foraging effort to multiple food sources in ants (Bonabeau et al. 1997), and nest selection and construction in ants (Franks et al. 2002; Pratt et al. 2002), bees (Seeley and Visscher 2004) and wasps (Jeanne 1975; Karsai and Penzes 1998) in search of general rules of how these systems might evolve. Comparative method such as those used here and elsewhere provides an additional and direct opportunity to examine how these complex behaviours evolve and the ecological and behavioural correlates that are associated with and underpin them, respectively, in order to examine both how these systems evolved and why.



Within the context of the evolution of nest-weaving in the ant genus *Polyrhachis*, complex behaviours can be readily lost and do not always follow an apparent transition from simple to complex. The additional insight from the ant genus *Polyrhachis* may simply be that as ants moved from arboreal down to subterranean habits, the pinnacle of cooperative behaviour, nest weaving, was not worth keeping.

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