Body size variation and caste ratios in geographically distinct populations of the invasive big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae)

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Body size is an important life history trait that can evolve rapidly as a result of how species interact with each other and their environment. Invasive species often encounter vastly different ecological conditions throughout their introduced range that can influence relative investment in growth, reproduction and defence among populations. In this study, we quantified variation in worker size, morphology and proportion of majors among five populations of a worldwide invasive species, the big-headed ant, *Pheidole megacephala* (Fabricius). The sampled populations differed in ant community composition, allowing us to examine if *P. megacephala* invests differently in the size and number of majors based on the local ant fauna. We also used genetic data to determine if these populations of *P. megacephala* represented cryptic species or if morphological differences could be attributed to change following introduction. We found significant variation in worker mass among the populations. Both major and minor workers were largest in Australia, where the ant fauna was most diverse, and minor workers were smallest in Hawaii and Mauritius, where *P. megacephala* interacted with few to no other ants. We also found differences in major and minor worker morphology among populations. Majors from Mauritius had significantly larger heads (width and length) relative to whole body size than those from Hawaii and Florida. Minors had longer heads and hind tibias in South Africa compared with populations from Australia, Hawaii and Florida. The proportion of majors did not differ among populations, suggesting that these populations may not be subject to trade-offs in investment in major size versus number. Our molecular data place all samples within the same clade, supporting that these morphologically different populations represent the same species. These results suggest that the variation in shape and morphology of major and minor workers may therefore be the result of rapid adaptation or plastic responses to local conditions. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **113**, 423–438.

ADDITIONAL KEYWORDS: competition – induced defences – invasion biology – life history – plasticity – trade-offs.

INTRODUCTION

Inducible defences are a primary strategy by which an organism can respond to immediate threats in its environment including predation, parasitism and competition (Harvell, 1990; Agrawal, 2001). Transgenerational induced defences, or adaptive maternal effects, are a method by which parents can improve their direct and indirect fitness by modifying the phenotype of their offspring in response to current

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environmental conditions (Agrawal, Laforsch & Tollrian, 1999). Maternally induced defences can include increases in chemical defensive compounds, the generation of specialized morphological structures (such as trichomes in plants or helmet and spine formation in *Daphnia*), or a general increase in investment in the number or size of offspring (Harvell, 1990; Repka & Pihlajamaa, 1996; Boersma, Spaak & De Meester, 1998; Agrawal, 1999; Agrawal *et al.*, 1999; Mondor, Rosenheim & Addicot, 2005; Kaplan *et al.*, 2008).

Body size is a key life history trait that is closely associated with an organism's physiology, behaviour, reproduction and survival. Size also influences how individuals interact with each other and their environment (Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984; Losos, 1990; Stearns, 1992; Brown, 1995; Moczek & Emlen, 2000; Chown & Gaston, 2010; Gouws, Gaston & Chown, 2011; Tökölyi, Schmidt & Barta, 2014). Environmental factors (e.g. resource availability, competitors), in turn, can shift reproductive investment in body size or alter an organism's developmental growth (Passera *et al.*, 1996; Moczek, 1998; Moczek *et al.*, 2002). Subsequently, body size is often measured in studies that examine trans-generational induced defences (Passera *et al.*, 1996; Boersma *et al.*, 1998; Van Buskirk, 2000; Relyea, 2004; Yang, Martin & Nijhout, 2004). Moreover, trade-offs exist in how limited resources are allocated to offspring size, number and condition (Smith & Fretwell, 1974), and these trade-offs may explain when organisms induce defence strategies (Harvell, 1990; Agrawal *et al.*, 1999; Van Buskirk, 2000).

While measuring investment in body size may be relatively straightforward in solitary organisms, in eusocial species, colonies partition investment into the number or quality of different castes. In ants, for example, a colony can invest in females by partitioning resources into discrete reproductive (queen) and non-reproductive (worker) castes. In species that have polymorphic workers, ant colonies can further differentially invest in sub-castes that may be specialized for food storage, foraging or defence (e.g. minors versus majors or soldiers) (Oster & Wilson, 1978; Hölldobler & Wilson, 1990; Kaspari & Byrne, 1995; Dornhaus & Powell, 2010). Castes in ants are determined by the amount and type of nutrition received during larval development (Wheeler, 1986; Hölldobler & Wilson, 1990; but see Heinze, 2008; Schwander *et al.*, 2010 for exceptions). The same is true for body size variation within the worker caste (Wheeler, 1991), although a number of recent studies have found evidence for a genetic influence on some size classes in polymorphic species (Hughes *et al.*, 2003; Jaffe *et al.*, 2007; Smith *et al.*, 2008; but see Wiernasz & Cole, 2010).

An advantage to environmental caste determination is that colonies can rapidly alter investment in the number and size of workers in response to biotic and abiotic conditions to optimize colony growth, maintenance, foraging and defence (Oster & Wilson, 1978; Wilson 1984; Beshers & Traniello, 1994; Kaspari & Byrne, 1995; Yang *et al.*, 2004; McGlynn, Diamond & Dunn, 2012). Both intra- and inter-specific competition, for example, may influence body size variation through foraging behaviour and prey selection (Davidson, 1978; Traniello, 1987, 1989; Wetterer, 1994). In the genus *Pheidole*, which has a dimorphic (and rarely trimorphic) worker caste consisting of majors and minors, colony investment in major production can be influenced by major size (Yang *et al.*, 2004; McGlynn *et al.*, 2012), diet supplementation (McGlynn & Owen, 2002), and in response to both intra- and inter-specific competition (Passera *et al.*, 1996; Yang *et al.*, 2004). Major workers generally specialize in nest defence and in food processing, retrieval and storage (Wilson, 1984). Minor workers typically undertake the remainder of tasks within the colony (Wilson, 1984, 2003). For example, Yang *et al.* (2004) found a greater percentage of majors in colonies of *Pheidole morrisi* from populations that overlapped with the red imported fire ant (*Solenopsis invicta*). However, the average size of these majors was smaller, suggesting that trade-offs existed between investment in major body size and major percentage/ratio. Shifts in caste ratio have also been observed in *Pheidole pallidula* where the perceived threat of competitors increased the production of major workers within laboratory colonies (Passera *et al.*, 1996). Differences in the competitive environment among populations may therefore induce shifts in investment into worker sub-castes that specialize in colony defence and resource acquisition.

In this study, we quantified variation in worker size, morphology and proportion of majors among five populations of a worldwide invasive species the big-headed ant, *Pheidole megacephala* (Fabricius, 1793). Introduced species often encounter dramatically different ecological conditions within their invaded ranges and this variation in selective pressures can impact relative investment into growth, reproduction and defence among introduced populations (e.g. Moczek *et al.*, 2002; Wolfe, 2002; Zangerl & Berenbaum, 2005; Berenbaum & Zangerl, 2006). We chose populations that differed in ant community composition allowing us to examine if *P. megacephala* invests differently in the size and number of workers based on its interactions with the local ant fauna. Specifically, we use our data to test two hypotheses: (1) populations introduced to areas with higher local ant diversity will produce either larger or a greater number of majors relative to populations introduced

to areas with few or no resident ants; and (2) populations will exhibit a trade-off between investment into major size and number. Finally, to determine if morphological differences among populations could be due to the presence of more than one 'cryptic' species, we use genetic data to examine the phylogenetic relationships among our populations relative to each other and to *P. megacephala* sequences available from the literature.

Our predictions specify changes in major production because major worker behavioural repertoires are often limited in *Pheidole* (Mertl & Traniello, 2009), showing greater specialization in resource acquisition and nest defence (Wilson, 1976, 2003). *Pheidole* colonies are also known to shift investment into major number or size in response to ecological conditions (Oster & Wilson, 1978; Kaspari & Byrne, 1995, Passera *et al.*, 1996; McGlynn & Owen, 2002; Yang *et al.*, 2004). Among our five sampled populations, we predict the greatest disparity in major production between colonies from Australia and Hawaii because the Hawaiian Islands have no native ant species (Wilson & Taylor, 1967) and Australia's Northern Territory is particularly species-rich (Andersen, 1997; Hoffmann, Andersen & Hill, 1999, Hoffmann & Parr, 2008) and include many competitively dominant ant species (Andersen, 1992). Larger regional species pools may also increase the probability of interaction with ecologically similar species and are thought to provide resistance to invasion (Elton, 1958; Simberloff, 1995; Stachowicz, Whitlatch & Osman, 1999; Kennedy *et al.*, 2002; Shea & Chesson, 2002). A trade-off between investment into major number and size among colonies would also suggest that colonies are resource-limited. Alternatively, developmental constraints on caste ratios could maintain stable major/minor proportions even when colonies are investing into larger majors. Together, these data will provide insight into how introduced species alter investment into defence when introduced to ecologically different environments.

MATERIAL AND METHODS
STUDY SYSTEM

As with nearly all *Pheidole* species, *P. megacephala* has a dimorphic worker caste consisting of minors and majors (also referred to as soldiers) that are easily distinguished by differences in size and head shape. Major workers have larger heads relative to their body size, and specialize in nest defence and in food processing, retrieval and storage (Wilson, 1984). Minor workers typically undertake the remainder of tasks within the colony (Wilson, 1984, 2003). We predicted that differences in ecological conditions

among geographically distinct populations (particularly the competitive environment) will result in variation in how colonies invest in worker body mass, morphology and proportion of majors.

We chose *P. megacephala* because it has successfully invaded a wide range of geographical locations (Wetterer, 2007, 2012) where different populations experience dramatically different ecological conditions. The native range of *P. megacephala* has not yet been determined although it is suspected to be from Africa (Ethiopian region) or Madagascar due to the richness of the *megacephala* species complex from these regions (Wheeler, 1922; Wetterer, 2007, 2012; Fischer, Garcia & Peters, 2012; Fournier *et al.*, 2012; Fischer & Fisher, 2013). We collected workers of *P. megacephala* from five geographically distinct populations that vary considerably in terms of species richness within the ant community: Northern Territory, Australia; Hawaii, USA; Florida, USA; Sabi Sands, South Africa; and Mauritius (Table 1). In each population, we sampled three to six nests and each nest was separated by between 200 m and 2 km. From each nest, we collected as many workers as possible by turning over a cover object that housed the nest, and aspirating as quickly as possible while also scooping ants into a container. All specimens were stored in 90% ethanol after collection.

Local ant community composition surrounding each invaded population was estimated by visual sampling and from the literature. This allowed us to estimate a range of ant species that *P. megacephala* had historically, or is currently, interacting with. The higher estimate represents the total pool of species within the region and the lower estimate represents the local species pool. For example, Hawaii has no native ant species (Wilson & Taylor, 1967), and the population of *P. megacephala* we sampled in Hawaii Volcanoes National Park had the potential to interact with 2–10 species (Wetterer, 1998, A. V. Suarez, pers. comm.). In contrast, tropical Australia has one of the most species-rich ant communities in the world. In our Howard Springs study area, 157 species have been recorded with the estimated number of ant species declining as the invasion progressed (Hoffmann *et al.*, 1999; Hoffmann & Parr, 2008). This site also has a number of species that are described as competitively dominant, notably green tree ants (*Oecophylla smaragdina*), meat ants (*Iridomyrmex* spp.) and other species that belong to the 'Dominant Dolichoderine' functional group (Hoffmann *et al.*, 1999). The estimate range of ant species in South Africa (34–121 species) (Parr & Chown, 2001; Parr, 2008), Florida (60–100 species) (Deyrup, 2003, M. Deyrup, pers. comm.) and Mauritius (8–16 species) (Smith & Fisher, 2009, A. V. Suarez, pers. comm.) fall between the estimated numbers for Hawaii and Australia. Species

Table 1. List of populations sampled for this study and characteristics for each population, including the year *P. megacephala* was first reported, the number of colonies sampled, climate and estimates of local ant community richness (native and introduced)

Population	Coordinates	First recorded	No. of colonies	Resident ant diversity
Australia, Howard Springs	12.49°S, 131.04°E	1887, 1996	4	21–157
South Africa, Sabi Sands	24.80°S, 31.50°E	1905	3	34–121
Florida, Sarasota	27.35°N, 83.53°W	1932	5	60–100
Mauritius	20.26°S, 57.55°E	1905	4	8–16
Hawaii, Volcanoes National Park	19.35°N, 155.47°W	1879	6	2–10

Dates of first record from Wetterer (2012); except Australia (taken from Hoffmann *et al*., 1999). For Australia we provide two dates, one for the continent and a second for its first detection in Howard Springs (Reichel & Andersen, 1996). South Africa is possibly within the native range of *P. megacephala* (but see discussion in Wetterer, 2012). Ant diversity estimate ranges are derived from our personal observations and from the following published accounts. The high end represents the total pool of species within the region and local estimates and the lower end estimates of local species pool from Hawaii (Wetterer, 1998; A. V. Suarez, pers. comm.), Australia (Hoffmann *et al*., 1999; Hoffmann & Parr, 2008), Florida (Deyrup, 2003; M. Deyrup, pers. comm.), South Africa (Parr & Chown, 2001; Parr, 2008) and Mauritius (Smith & Fisher, 2009; A. V. Suarez pers. comm.).

pool estimates from all sites, with the exception of Florida, were derived from species surveys conducted within 50 km of sampling locations. Species pool estimates from Florida are derived from surveys conducted < 200 km from sampling locations. We examined if investment into worker size or proportion of majors is related to the regional diversity of resident species pools at each site because we lack information on the identity of most ant species interacting with *P. megacephala* at each site (including their colony size, dominance and ecological overlap).

PHYLOGENETIC ANALYSIS

DNA isolation

Field collections were made in 90–95% EtOH and kept in the laboratory until the time of DNA extraction. Thirty-three new specimens of *P. megacephala* were sequenced for this study (Table 2). Total genomic DNA was isolated for one individual worker by first pulverizing with a tungsten carbide bead in a TissueLyser (Qiagen), followed by purification using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocols.

Polymerase chain reaction (PCR) amplification

For most specimens, four fragments were amplified via PCR using specific primers for each gene region following the protocols of Moreau *et al*. (2006) and Moreau (2008): *cytochrome oxidase* I (COI) protein encoding mitochondrial marker, *long-wavelength rhodopsin* (LR) protein encoding nuclear marker, *H3 histone* (H3) protein encoding nuclear marker, and

12S mitochondrial ribosomal DNA marker, for a total of almost 2300 bp of aligned sequence.

Sequencing

All sequencing was done using dye terminator cycle sequencing using BigDye terminator v3.1 and an ABI 3730 DNA analyser (Life Technologies). Primers used for amplification served as sequencing primers. All samples were sequenced in both directions again following the protocols of Moreau *et al*. (2006) and Moreau (2008).

Sequence alignment

In addition to the new sequence data collected for 33 new specimens, we incorporated 56 additional relevant sequences generated for other studies (Moreau, 2008; Smith & Fisher, 2009; Fournier *et al*., 2012) for a total of 89 taxa included in this study (Table 2). After sequence data were collected they were analysed and initially aligned using the computer program Geneious v6.1.2 (Drummond *et al*., 2012). Inferred amino acid sequences were used for all protein-coding genes, allowing for comparatively uncomplicated alignment using Mesquite v2.75 (Maddison & Maddison, 2011).

Phylogenetic analysis

Our phylogenetic approach is used to confirm that our sampled populations belong to the same species, not to infer relationships among introduced populations of *P. megacephala* (which would require a large number of diverse markers). To infer relationships among the species of *Pheidole* included in this study, several model-based phylogenetic analyses were performed on the CIPRES Science Gateway

Table 2. List of *P. megacephala* specimens, collection accession numbers, collection locality, and GenBank accession numbers

Accession no.	Locality	mtDNA CO1	mtRNA 12S	nDNA LR	nDNA H3
AVS 1809	Hawaii	KF171399	KF171368	X	KF171430
AVS 1810	Hawaii	KF171400	KF171369	X	KF171431
AVS 1811	Hawaii	KF171401	KF171370	X	KF171432
AVS 1812	Hawaii	KF171402	KF171371	X	X
AVS 1813	Hawaii	X	X	X	KF171433
AVS 1814	Hawaii	KF171403	KF171372	X	X
AVS 1823	Hawaii	KF171404	KF171373	X	KF171434
AVS 1848	Hawaii	KF171405	KF171374	X	KF171435
AVS 2625	Mauritius	KF171410	KF171379	KF171460	KF171440
AVS 2632	Mauritius	KF171411	KF171380	X	KF171441
AVS 2647	Mauritius	KF171412	KF171381	KF171461	KF171442
AVS 2659	Mauritius	KF171413	KF171382	KF171462	KF171443
AVS 2694	Mauritius	KF171414	KF171383	KF171463	KF171444
AVS 2695	Mauritius	KF171415	KF171384	KF171464	KF171445
AVS 2717	Mauritius	KF171416	KF171385	X	X
BD 10	Missouri	KF171417	KF171386	KF171465	KF171446
BD 3	Missouri	KF171418	KF171387	KF171466	KF171447
BD 5	Missouri	KF171419	KF171388	KF171467	KF171448
BD 9	Missouri	KF171420	KF171389	KF171468	KF171449
BFL13	Florida	KF171392	KF171361	X	KF171423
BFL14	Florida	KF171393	KF171362	KF171453	KF171424
BFL15	Florida	KF171394	KF171363	KF171454	KF171425
BFL16	Florida	KF171395	KF171364	X	KF171426
BFL17	Florida	KF171396	KF171365	X	KF171427
CSM1381	Florida Keys	KF171397	KF171366	KF171455	KF171428
CSM1403	Florida Keys	KF171398	KF171367	KF171456	KF171429
CSM2617	Uganda	KF171422	KF171391	KF171469	KF171452
HS1	Australia	KF171406	KF171375	KF171457	KF171436
HS2	Australia	KF171407	KF171376	X	KF171437
HS3	Australia	KF171408	KF171377	KF171458	KF171438
HS4	Australia	KF171409	KF171378	KF171459	KF171439
Sabi Sands 2	S. Africa	X	X	X	KF171450
Sabi Sands 3	S. Africa	KF171421	KF171390	X	KF171451

(Miller, Pfeiffer & Schwartz, 2010) using RAxML v7.3.2 (Stamatakis, Ludwig & Meier, 2005) and MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001). To evaluate the fit of the data, likelihood analyses were conducted using both the COI-only data set (COI-only) and the data partitioned by individual genes (partitioned). Modeltest 3.06 (Posada & Crandall, 2001) was used to determine the most appropriate nucleotide substitution model. Two maximum-likelihood searches were implemented in RAxML: (1) a single model of sequence evolution was assumed to underlie the mtDNA COI gene (COI-only) with 500 bootstrap pseudoreplicates and (2) one that allowed each of the four gene regions to have a separate model of sequence evolution with parameters unlinked (partitioned) with 500 bootstrap pseudoreplicates.

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 (BPPs) were estimated as the proportion of trees sampled after 10% burn-in that contained each of the observed bipartitions (Rannala & Yang, 1996; Larget & Simon, 1999). Again, two analyses were performed: (1) a single model of sequence evolution was assumed to underlie the mtDNA COI gene (COI-only) and (2) one that allowed each of the four gene regions to have a separate model of sequence

evolution with parameters unlinked (partitioned). Independence of runs was ensured by accepting only analyses where the average standard deviation of split frequencies was below 0.01.

BODY MASS AND MORPHOLOGY

We collected as many workers as possible from multiple nests within each population (see Table 1). From each nest, 25 minor workers and as many major workers as available (mean ± SE 8.82 ± 1.22, range 3–20) were dried in an oven at 50 °C for ~48 h. After 48 h, we placed workers into 1.5-mL microcentrifuge tubes (to prevent re-hydration) and then weighed each individual using a UMX2 microbalance with 0.1-µg resolution (Mettler-Toledo). After weighing, each specimen was point mounted and head length (HL), head width (HW), pronotal width (PW) and hind tibia length (TL) were measured using a Semprex Micro-DRO digital stage micrometer (0.005-mm resolution, Semprex) connected to a Leica MZ 12.5 stereomicroscope.

To examine how mean body mass and morphological measurements differed among populations, we first selected models in R v2.15.1 (R Core Team, 2014) (see Crawley, 2012) with the R package lme4 (Bates, Maechler & Bolker, 2013). We compared the mean measurement per colony among populations with colonies as replicates with each worker as a subsample of colony. Majors and minors were identified a priori as workers are completely dimorphic and worker sub-caste was easily distinguished based on overall size and the shape of the head. For both sets of workers, and for all measurement data, the first model included population as a fixed effect and colony as random effect. We found no significant effect of colony identity within sites ($P > 0.99$), and thus we only included population as a fixed effect for analysis. We compared among-population variation in body mass for each caste using an analysis of variance (ANOVA) and then used a post-hoc Tukey's honestly significant difference (HSD) correction to determine which populations were different from each other. We compared morphological measurements among populations for each caste using a principal components analysis (PCA) and compared differences between significant principal components with an ANOVA and post-hoc Tukey HSD.

We used logistic regressions of body mass (continuous variable) on caste (1 for major, 0 for minor) to generate clines to examine body size thresholds that distinguish majors from minors in each population. To estimate each colony threshold we used dose.p in R v2.15.1 (R Core Team, 2014) to estimate the mass at which a worker is equally likely to be assigned to a major or minor. As with measurement data, we first selected a model including colony as a random effect but found no effect of colony ($P > 0.76$), and thus we included only population as a fixed effect. We compared variation among populations in colony thresholds using an ANOVA and then used a post-hoc Tukey's HSD correction to determine which populations were different from each other.

PROPORTIONS OF MAJORS

To compare the proportion of majors from each population, we counted the total number of majors and minors sampled from each nest, and calculated the proportion of majors within a colony. We compared proportions of majors between populations in R v2.15.1 (R Core Team, 2014) using a binomial distribution with a generalized linear model (GLM) with the R package lme4 (Bates *et al*., 2013). The original model was over-dispersed, so we refit the model with quasi-binomial to account for the over-dispersion (see Crawley, 2012).

RESULTS

SIMPLE SEQUENCE STATISTICS

This study produced a final aligned 2282-bp fragment with the following four gene regions: a fragment spanning the mitochondrial *cytochrome oxidase* I (COI) (1054 bp) gene, a fragment of the mitochondrial ribosomal DNA marker *12S* (349 bp), a portion of the nuclear protein-encoding gene *long-wavelength rhodopsin* (LR) (555 bp) and a fragment of the nuclear protein-encoding gene *H3 histone* (H3) (324 bp). The aligned fragment contained 1351 constant sites (59.3%), 264 uninformative variable sites (11.5%) and 667 parsimoniously informative sites (29.2%). Most specimens were sequenced for all four genes with the following exceptions: COI missing for two taxa (AVS1813, SabiSands2), LR missing for 16 taxa (see Table 2), 12S missing for two taxa (AVS1813, SabiSands2) and H3 missing for three taxa (AVS1812, AVS1814, AVS2717) (Table 2).

PHYLOGENETIC ANALYSES

The maximum-likelihood topology for the COI-only analysis is presented in Figure 1 with maximum-likelihood bootstrap (ML BS) support values for both the COI-only and partitioned analyses, as well as the BPP support values for both the COI-only and partitioned analyses included. A GTR + G model of sequence evolution was found to be the best fit to the data for all partitions. All maximum-likelihood and Bayesian inference tree topologies show strong support (100% ML BS COI-only; 87% ML BS partitioned; 1.0 BPP COI-only; 1.0 BPP partitioned) for the
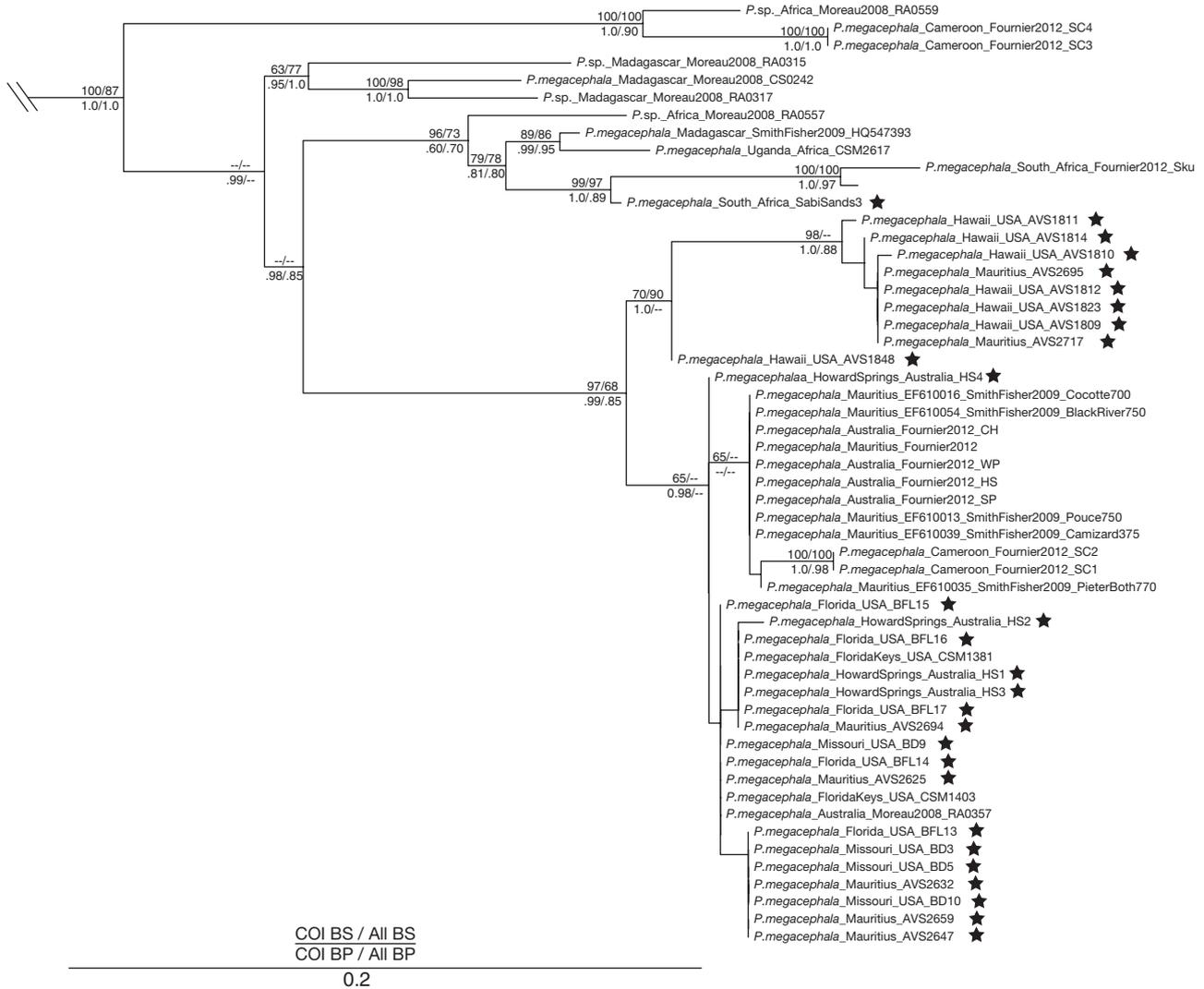
Figure 1. Phylogram of *Pheidole megacephala* as inferred through maximum-likelihood analysis for the COI dataset. Collections of *P. megacephala* measured for size, morphology and proportion of majors as part of this study are noted by a star next to the taxa names. Branch lengths are proportional to substitutions per site as indicated by the bottom legend inset. Clade support greater than 50% is denoted on branches as follows: values above branches represent maximum-likelihood bootstrap (ML BS) for the COI-only dataset followed by the partitioned dataset and values below branches represent Bayesian posterior probabilities (BPPs) for the COI-only dataset followed by the partitioned dataset. Clade support of '--' denotes clades not supported in an individual analysis. Taxa names include taxonomic identity, state and country of collection site, and collector code (and GenBank accession number and citation to original publication if from a previous study).

monophyly of the *P. megacephala* samples from this study with previously sampled specimens from Moreau (2008), Smith & Fisher (2009) and Fournier *et al*. (2012).

BODY MASS AND MORPHOLOGY

The body mass of majors differed significantly among populations (ANOVA $F_{4,17} = 29.73$, $P < 0.0001$) (Fig. 2A). Majors from Australia were larger than those in other comparisons (Tukey HSD post-hoc p-adj < 0.001), but no other populations were different from one another. The body mass of the minors also differed significantly among populations (ANOVA $F_{4,17} = 9.47$, $P < 0.0001$) (Fig. 2B). The minors were larger in Australia compared with minors from the other four populations (Tukey HSD post-hoc p-adj < 0.001). Minors from South Africa and Florida were also larger than minors from Mauritius and Hawaii (Tukey HSD post-hoc p-adj < 0.05).
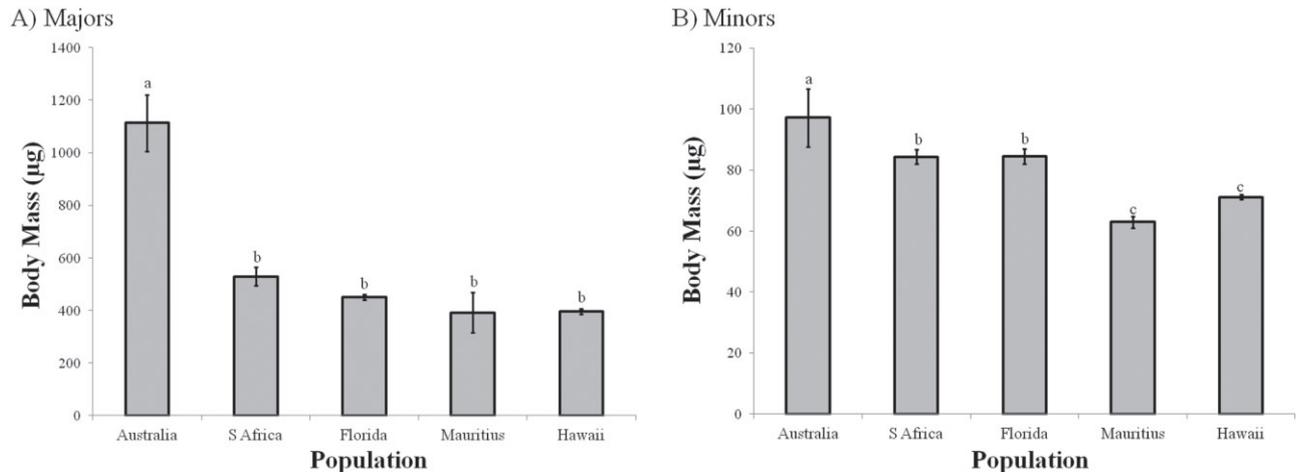
A) Majors

B) Minors

Figure 2. Variation in mean body mass of (A) majors and (B) minors among populations of *Pheidole megacephala*. Bars represent mean ± SE body mass per colony and letters indicate differences between populations (Tukey HSD post-hoc p-adj < 0.05). A, the body mass of majors differed significantly among populations (ANOVA $F_{4,17}$ = 29.73, $P < 0.0001$). Majors from Australia were larger than those in other comparisons (Tukey HSD post-hoc p-adj < 0.001). B, the body mass of the minors differed significantly among populations (ANOVA $F_{4,17}$ = 9.4662, $P < 0.0001$) and minors were larger in Australia compared with minors from Hawaii and Mauritius (Tukey HSD post-hoc p-adj < 0.001), and minors from Mauritius were larger than those collected in South Africa (Tukey HSD post-hoc p-adj < 0.05).

Table 3. The eigenvectors calculated from the original morphological measurements and the percentage contribution of components to observed variation in major and minor workers

Principal components	PC1	PC2	PC3	PC4
Majors				
Head length	−0.7075	0.7002	−0.0237	−0.0926
Head width	−0.6329	−0.6822	−0.2627	−0.2549
Pronotal width	−0.2768	−0.1508	0.1492	0.9372
Tibia length	−0.1487	−0.1470	0.9530	−0.2193
Proportion of variance	0.7873	0.1098	0.0569	0.0464
Cumulative proportion	0.7873	0.8971	0.9536	1.0000
Minors				
Head length	0.5632	0.5319	−0.4763	0.4160
Head width	0.7127	0.0737	0.5416	−0.4394
Pronotal width	0.1708	−0.3095	−0.6927	−0.6286
Tibia length	0.3814	−0.7847	0.0001	0.4886
Proportion of variance	0.5302	0.1733	0.1547	0.1409
Cumulative proportion	0.5302	0.7074	0.8591	1.000

Bold type indicates morphological measurements are closely associated (> |0.5|) with principal components.

For majors, PC1 explained 79% of the variation in the morphological measurements and was primarily associated with head length and head width (Table 3). Populations differed with respect to PC1 values (ANOVA $F_{4,17}$ = 6.96, $P < 0.01$) with majors from Mauritius having longer and wider heads than majors from Florida and Hawaii (Tukey HSD post-hoc p-adj < 0.001) (Fig. 3A). For minors, PC1 explained 53% and PC2 explained 17% of the variance in morphology (70% overall). As in majors, PC1 was associated with variation in head length and head width. PC2 also included variation in hind tibia length. Unlike in majors, PC1 was not informative in distinguishing minors from any population (ANOVA $F_{4,17}$ = 1.98, $P = 0.14$). However, there was a difference among populations in the shape of minors using PC2 (ANOVA $F_{4,17}$ = 20.6, $P < 0.0001$). Minors from South Africa had longer heads and hind tibia length than those in Australia, Hawaii and Mauritius (Tukey HSD post-hoc p-adj < 0.05) and minors from Australia

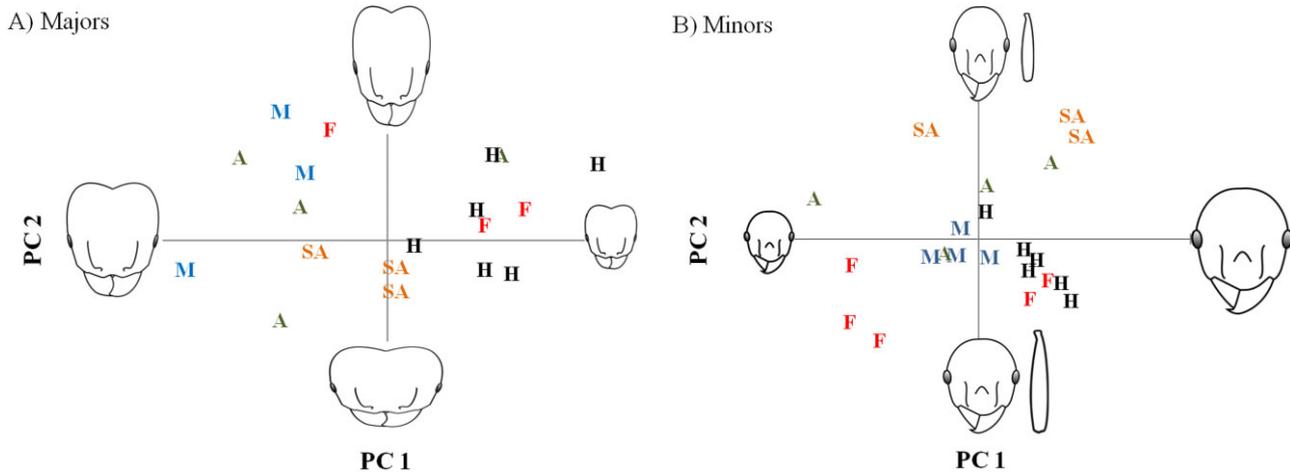A) Majors

B) Minors

PC 2

PC 1

Figure 3. PCA of (A) majors and (B) minors among populations (Australia, green, A; Florida, red, F; Hawaii, black, H; Mauritius, blue, M; South Africa, orange, SA) of *Pheidole megacephala*. For majors, PC1 explained 79% of the variation in the morphological measurements and was primarily associated with head length and head width. Populations differed with respect to PC1 values (ANOVA $F_{4,17} = 6.96$, $P < 0.01$) with majors from Mauritius being longer and wider than majors from Florida and Hawaii (Tukey HSD post-hoc p-adj < 0.001). For minors, PC1 explained 53.01% and PC2 explained 17% of the variance in morphology (70% overall). As in majors, PC1 was associated with variation in head length and head width. PC2 also included variation in hind tibia length. For minors, PC1 was not informative in distinguishing minors from any population (ANOVA $F_{4,17} = 1.98$, $P = 0.14$). However, there was a difference among populations in the shape of minors using PC2 (ANOVA $F_{4,17} = 20.6$, $P < 0.0001$). Minors from South Africa have longer heads and hind tibia length than those from Australia, Hawaii and Mauritius (Tukey HSD post-hoc p-adj < 0.05) and minors from Australia were different from those from Florida and Hawaii (Tukey HSD post-hoc p-adj < 0.05).

were different from those from Florida and Hawaii (Tukey HSD post-hoc p-adj < 0.05) (Fig. 3B).

From the isoclines (Fig. 4), we can visually represent the critical weight at which workers transition from minors to majors. The body size thresholds that separate minor from major workers differed significantly among populations (ANOVA $F_{4,17} = 6.14$, $P = 0.003$). The post-hoc tests revealed that the critical weight at which workers transition from minors to majors was larger in Australia than all other populations (Tukey HSD post-hoc p-adj < 0.05).

PROPORTION OF MAJORS

The proportion of majors within a colony was not significantly different among populations (ANOVA $F_{4,17} = 2.67$, $P = 0.0619$) although there was a trend for nests in Florida to have fewer majors relative to the other four populations: Australia, 0.21 ± 0.1 (mean ± SE); South Africa, 0.25 ± 0.06; Florida, 0.06 ± 0.01, Mauritius, 0.19 ± 0.01; and Hawaii, 0.19 ± 0.06 (Fig. 5).

DISCUSSION

Ants are interesting model organisms for the study of inducible defences as extensive overlap among genera-

tions allows queens to directly benefit from differential investment into offspring condition. Moreover, in addition to maternally induced effects (from queen provisioning of the egg or larvae), in mature colonies distributed effects operate through differences in nutritional regimes towards larvae that result from variation in nursing and foraging behaviour of workers. Previous work has suggested that competition for resources (Davidson, 1978; Traniello, 1987, 1989; Wetterer, 1994) and perceived threats (Passera et al., 1996; Yang et al., 2004) may influence colony investment into worker body size and caste ratios. Invasive species can encounter different competitive environments throughout their ranges (Holway et al., 2002; Moczek et al., 2002; Wolfe, 2002; Zangerl & Berenbaum, 2005; Berenbaum & Zangerl, 2006) and may be particularly good models to examine how colonies invest into defence. Because of the more specialized roles of major workers (Wilson, 1984, 2003), we predicted that the invasive *P. megacephala* would increase investment into major size or number in populations introduced to areas with high local ant diversity relative to populations introduced to areas with few or no ant species. In addition, if a trade-off exists between investment into the number and size of major workers, then we predict that populations that invest more into larger majors will also produce fewer majors.
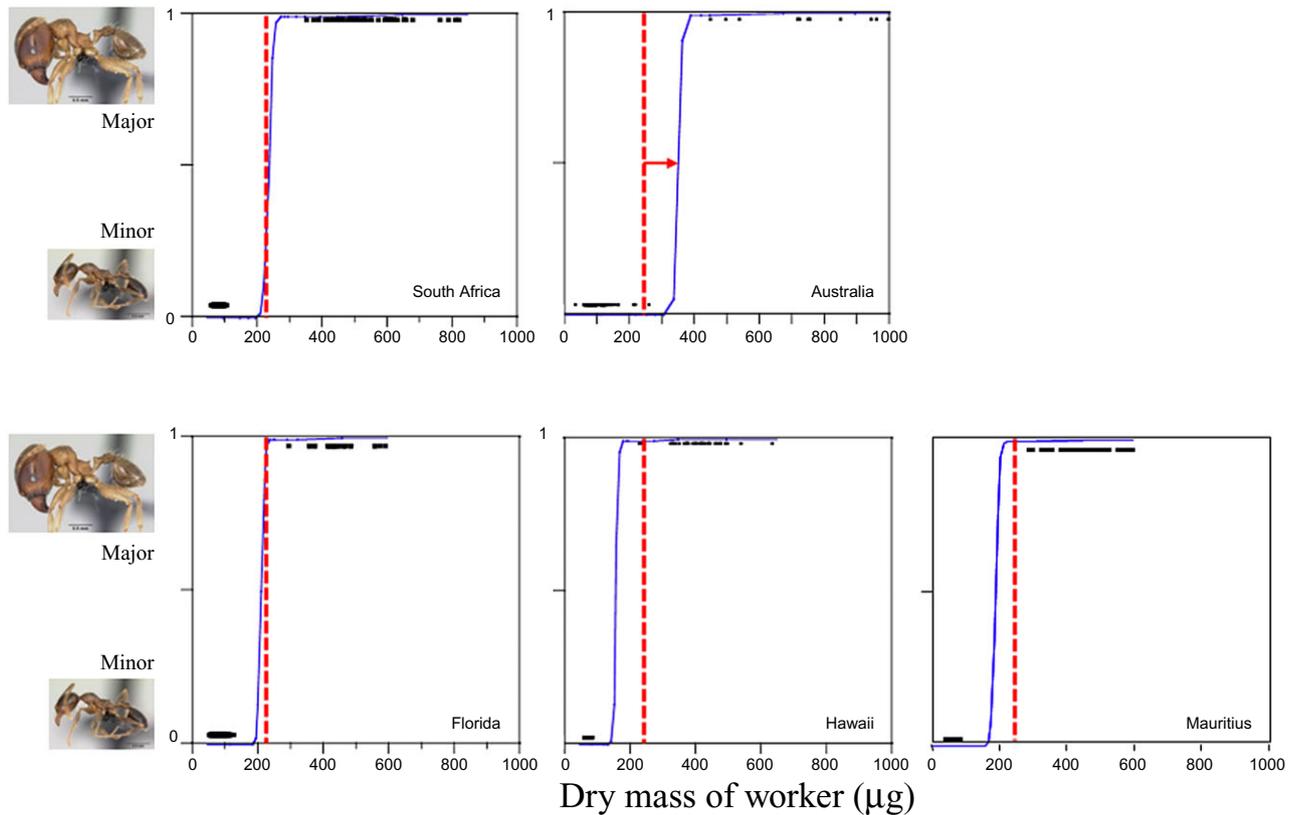
Figure 4. Isoclines of major and minor thresholds among populations of *Pheidole megacephala*. The body size threshold differed significantly among populations (ANOVA $F_{4,17} = 6.14$, $P = 0.003$) and the Australian threshold was larger than all other populations (Tukey HSD post-hoc p-adj < 0.05). The vertical dashed line in each graph represents the mean threshold among all populations. Photos: Antweb.org
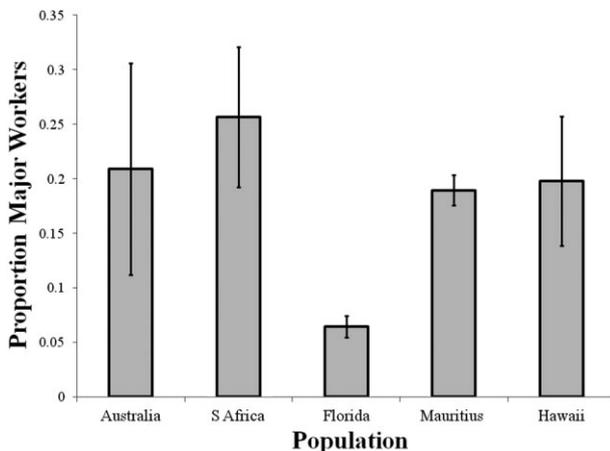

Figure 5. Mean (± SE) of worker caste distributions of *Pheidole megacephala* among populations. The proportion of major workers represents the number of majors divided by the total number of ants collected. The proportion of majors within a colony was not significantly different among populations (ANOVA $F_{4,17} = 2.67$, $P = 0.06$).

We found substantial variation in how colonies invest into worker mass and morphology among five populations of the invasive ant *P. megacephala*. Both major and minor workers were largest in Australia, and minor workers were smallest in Hawaii and Mauritius. Head shape also varied among populations in both worker sub-castes. Our genetic results suggest that these populations are nominally all the same species. Subsequently, differences into investment among populations in the size of majors probably result from rapid adaptation or a plastic response to local conditions. While we only sampled ants from five different countries, these populations varied considerable in ecological conditions and we discuss our results in the context of this ecological variation.

Our preliminary phylogeny placed ants from all five populations within a clade that included most previously published sequences from populations identified as *P. megacephala*. However, recent molecular evidence (Moreau, 2008; Fournier *et al.*, 2012) suggests that the *P. megacephala* complex in Africa and

Madagascar is taxonomically unresolved and that there may be more than one cryptic species introduced from these regions that is nominally called *P. megacephala*. The presence of cryptic species can present challenges to the study of invasions from a number of perspectives. First, they can hinder efforts to identify the source of introduction and effective biological control agents. Second, cryptic species can mislead researchers to believe large variation in ecological, behavioural and genetic characteristics exists among populations of the same species when it is actually separate species that are being compared. This has been an issue with a number of ant species (e.g. *Solenopsis invicta/S. richteri* – Wilson, 1951; *Tetramorium caespitum/T. tsushimae* – Steiner *et al.*, 2006; *Technomyrmex albipes/T. difficilis* – Bolton, 2007). In the case of *P. megacephala*, we need considerably more work examining genetic variation among introduced populations and also across its putative native range. Without this information, it will be impossible to identify the geographical origin of this widespread species and conclusively determine if multiple, related taxa may have been introduced. In principle, this can only be done with an extensive and careful taxonomic revision of this species group in Africa and Madagascar (*sensu* Fischer *et al.*, 2012; Fischer & Fisher, 2013).

Our results support one of our two predictions: that populations of *P. megacephala* introduced to areas with high native ant diversity would invest more into majors. Specifically, we found that both majors were larger in Australia than in other populations. Although many studies have quantified body size variation among species (Cushman, Lawton & Manly, 1993; Beshers & Traniello, 1994; Kaspari & Byrne, 1995; Kaspari, 2005; Geraghty, Dunn & Sanders, 2007; McGlynn *et al.*, 2012), few examine how variation differs across wide geographical locations within a single species. Our results correspond well with previous studies examining geographical variation in worker body size in *Pheidole*. In a study of three populations of *Pheidole morrisi* in the United States, Yang *et al.* (2004) found colonies from Florida produced significantly more, smaller majors in Florida than colonies from New York and New Jersey. Populations in New York and New Jersey experience longer, colder winters than colonies in Florida, whereas colonies in Florida encounter the aggressive red imported fire ant (*Solenopsis invicta*). In addition to differences in major size, both major and minor workers from New York and New Jersey stored more fat than those collected in Florida (Yang, 2006). Unfortunately, we were unable to examine fat content in our samples because our specimens were stored in 90% ethanol.

We also found minor workers from Australia to be larger than minors from other populations, and colo-

nies from Hawaii and Mauritius had the smallest minor workers. While we did not make any a priori predictions about minor worker size, minor workers also are important components in colony defence and resource retrieval (Detrain & Pasteels, 1991, 1992). These patterns suggest a change in the size of majors is accompanied by a similar change in minors due to either (1) a shift in the threshold at which larvae commit to caste-specific developmental pathways (Wheeler, 1991; Moczek, 1998) or (2) the growth period after the developmental switch has been extended pre-pupation. This pattern warrants further exploration and would benefit from employing developmental approaches (e.g. Rajakumar *et al.*, 2012). Moreover, future examinations of how *P. megacephala* colonies invest into worker size and condition, at small spatial scales in relation to interactions with resident ant species, will probably uncover patterns missed at larger scales as in our study.

The relative shape of both majors and minor workers also varied among populations. Majors from Mauritius had significantly larger heads (width and length) than those from Hawaii and Florida. In minor workers, the length of the head and hind tibia were greater in South Africa relative to minors from Australia, Hawaii and Florida. These results suggest that differences in body mass do not necessarily translate to predictable differences in morphology and shape. Most morphological variation among species of *Pheidole* can be attributed to size differences among species (Pie & Traniello, 2007). However, variation in morphology between castes may reflect differences in selective pressures based on their function (Pie & Traniello, 2007). Prior work has shown that the interaction between phylogenetic history and ecology induces interspecific variation in *Pheidole* species in the Neotropics, where interspecific variation in major worker size and morphology is tightly related to habitat type (ground- or twig-nesting), foraging strategy (discovery versus dominance), and major worker behaviour (major worker recruitment to food resources) (Mertl, Sorenson & Traniello, 2010). Variation in shape among populations in our study suggests a response to local ecological conditions separate from selection on overall size. For example, eco-morphological studies have shown that head shape and leg length can influence foraging behaviour, trophic position, community assembly and how ants can navigate complex topography (e.g. size-grain hypothesis) (Traniello, 1989; Kaspari & Weiser, 1999; Weiser & Kaspari, 2006; Silva & Brandão, 2010). The observed intraspecific variation in shape and morphology of major and minor workers may therefore be the result of rapid adaptation or plastic responses to widely varying local conditions.

We did not find support for our second prediction that the proportion of majors would vary among

populations. The caste ratios we report across populations of *P. megacephala* (0.06–0.26) is similar to the range reported across species of Neotropical *Pheidole* (0.05–0.20: Kaspari & Byrne, 1995; 0.02–0.27: McGlynn *et al*., 2012). If resources are limiting, colony investment into the size and number of workers could be subject to trade-offs, so that a colony producing larger majors may produce fewer of them (Yang *et al*., 2004; Mertl & Traniello, 2009; McGlynn *et al*., 2012). Major production can also vary seasonally, for example in response to the production of sexual larvae (Johnston & Wilson, 1985; Kaspari & Byrne, 1995; Brown & Traniello, 1998). Moreover, the presence of soldiers in a colony can inhibit further soldier production, thereby creating stable minor/major ratios (Wheeler & Nijhout, 1984). Our results may reflect this general constraint although this seems unlikely given patterns seen in other *Pheidole* species where the number of majors increases in response to changes in diet or the competitive environment (Passera *et al*., 1996; McGlynn & Owen, 2002; Yang *et al*., 2004). In this study, we employed the same sampling method at each site, allowing us to compare the proportions of majors among populations. However, our sampling design may have been insufficient to accurately estimate the proportion of majors in each nest. To better estimate worker demography, greater effort is required to collect the entire colonies. However, *P. megacephala* colonies are difficult to collect in their entirety because they are extremely polydomous. Future efforts to estimate worker proportions or caste ratios should include quantifying the ratio of majors that respond to a specific stimulus to the colony such as access to a bait or threat for competitors, by counting the number of soldiers that show up to baits at fixed distances to the colony (Huang, 2010; Mertl *et al*., 2010).

In addition to competition, diet (McGlynn & Owen, 2002) and climate (Yang *et al*., 2004) also influence colony investment into worker body size in *Pheidole* species. Shifts in worker size may result from a release in resource limitation by accessing resources neglected by native species (see Shea & Chesson, 2002). With our sampling design, we were unable to explicitly test the contribution of each of these factors separately. Future work should sample more populations across a wider range of biotic and abiotic conditions. Furthermore, field surveys should be combined with common-garden experiments to explore the relative influence of each factor on the morphology of workers. Despite the limitations of the current study, our results serve as a foundation for the testing of specific hypotheses. For example, although we did not directly test the influence of competition on worker body size, we do see a pattern that suggests a relationship between competitive environment and

major worker body size. Further work is needed to decipher the role of diet, climate and competition on the observed variation in worker body sizes.

The interplay of body size and invasion success has been investigated in a biogeographical context in a variety of taxa (plants: Crawley, 1987; Thébaud & Simberloff, 2001; vertebrates: Veltman, Nee & Crawley, 1996; Jeschke & Strayer, 2006; Blackburn *et al*., 2013; invertebrates: Lawton *et al*., 1986; McGlynn, 1999; Miller, Hewitt & Ruiz, 2002; Roy, Jablonski & Valentine, 2002). In fact, differential investment into defence, growth or reproduction between native and introduced populations forms the basis for many hypotheses as to why introduced species are so successful in invaded areas (Blossey & Notzhold, 1995; Keane & Crawley, 2002; Shea & Chesson, 2002; Parker *et al*., 2013). Much research in this area examines whether species are larger in introduced populations relative to populations in their native range (Parker *et al*., 2013). While the data supporting the role of body size and invasion success is generally mixed, introduced species of insects are often smaller than their native counterparts (Lawton *et al*., 1986; McGlynn, 1999). Additionally, some introduced ant species are known to be smaller within their introduced range than in their native range (McGlynn, 1999; Mikheyev & Mueller, 2007). In social insects, trade-offs between worker size and worker number may promote the success of invaders by either promoting larger individuals that will do well in one-on-one encounters, or by allowing species to obtain the high worker densities needed to displace resident species by outnumbering them with smaller individuals (Franks & Partridge, 1993). Understanding how body size both responds and contributes to establishment success in new areas promises to continue to be an important area of research for invasion biology and ecology. Of particular interest will be future studies that combine evolutionary–developmental approaches to study variation in size and shape (Abouheif & Wray, 2002; Moczek *et al*., 2002; Rajakumar *et al*., 2012) with ecological studies of the consequences of this variation.

ACKNOWLEDGEMENTS

for Molecular Systematics and Evolution at the Field Museum of Natural History, Chicago, Illinois and supported in part by a National Science Foundation grant (NSF DEB 1050243) to C.S.M. All remaining portions of this research were supported by grants from the National Science Foundation (NSF DEB 1020979) to A.V.S. and the University of Illinois, Department of Animal Biology, to B.D.W. Thanks to the CSIRO OCE Visiting Scientist Program. Finally, we thank three anonymous reviewers for comments that greatly improved the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Full phylogram of *Pheidole megacephala* as inferred through maximum-likelihood analysis for the COI dataset. A portion of the phylogram presented in Figure 1 is denoted by the dashed box. Collections of *P. megacephala* sequenced as part of this study are noted by a star next to the taxa names. Branch lengths are proportional to substitutions per site as indicated by the bottom legend inset. Clade support greater than 50% is denoted on branches as follows: values above branches represent maximum-likelihood bootstrap (ML BS) for the COI-only dataset followed by the partitioned dataset and values below branches represent Bayesian posterior probabilities (BPPs) for the COI-only dataset followed by the partitioned dataset. Clade support of '--' denotes clades not supported in an individual analysis. Taxa names include taxonomic identity, state and country of collection site, and collector code (and GenBank accession number and citation to original publication if from a previous study).

Table S1. List of *Pheidole* spp. identity, collection accession numbers, collection locality, and GenBank accession numbers for four genes for all specimens included in our phylogeny (Figure S1).