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# Population and colony structure and morphometrics in the queen dimorphic harvester ant, *Pogonomyrmex pima*

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**Abstract.** The North American seed-harvester ant *Pogonomyrmex* (*Ephebomyrmex*) *pima* displays a dimorphism that consists of winged (alate) and wingless (intermorph) queens; both types of queens are fully reproductive. Microsatellite allele frequencies and a mitochondrial phylogeny demonstrate (1) alate and intermorph queens represent an intraspecific wing polymorphism, and (2) an absence of assortative mating and inbreeding by males. Surveys at our field site in southcentral Arizona, USA, demonstrated that only one type of queen (intermorph or dealate) occurred in each colony, including those excavated during the season in which reproductive sexuals were present. Colony structure appeared to vary by queen type as most intermorph colonies contained multiple mated queens. Alternatively, dealate queen colonies rarely contained a mated queen. Our inability to find mated dealate queens in these colonies probably resulted from difficulty in excavating the entire colony and reproductive queen, especially given that these colonies were only excavated over one day.

A morphometric analysis demonstrated that intermorph queens are intermediate in size to that of workers and alate queens, but that intermorph queens retain all of the specialized anatomical features of alate queens (except for wings). Some colonies had queens that foraged and performed nest maintenance activities, and these queens sometimes accounted for a significant portion of colony foraging trips. Dissections revealed that these queens were uninseminated; some of these queens produced males in the laboratory.

*Keywords:* Intermorph queen, lineage analysis, morphometrics, polyphenism, queen dimorphism, queen foraging.

## Introduction

Dispersal polymorphisms have played a significant role in our understanding of population dynamics, dispersal dynamics, life history, and the physiological basis of adaptation (Zera and Denno, 1997). Such polymorphisms are common among insects, and typically consist of one form that is fully capable of flight and a second form that is flightless. At the proximate level, these dimorphisms result from variation in genotype, environment, or from a combination of both; at the ultimate level, flight ability is associated with habitat persistence. Flight allows organisms to disperse from heterogeneous or temporary habitats, while flightlessness promotes survival in homogeneous or persistent habitats (Heinze and Tsuji, 1995; Roff, 1986,1990; Zera and Denno, 1997).

Within species of ants, reproductive females (queens) exhibit two types of dispersal polymorphisms (Heinze and Keller, 2000; Peeters and Ito, 2001; Rüppell and Heinze, 1999). One type involves a size dimorphism in which both queen forms possess wings and are capable of flight, i.e., macrogynes and microgynes. The second type of dimorphism involves presence or absence of wings, i.e., winged (alate) queens versus wingless ergatoid or intermorph queens. In both cases, the two queen forms typically exhibit different colony founding strategies. Macrogynes and winged queens usually disperse from colonies to mate and found colonies independently, whereas their small-bodied (microgyne) or wingless (ergatoid or intermorph) counterparts have limited ability to disperse, and employ dependent colony founding via (re)adoption into established colonies (McInnes and Tschinkel, 1995; Rüppell et al., 2001a). The one known exception to this pattern is that both macrogyne and microgyne queens of *Ectatomma ruidum* (subfamily Ectatomminae) can successfully initiate colonies independently by foraging during the founding period (Schatz et al., 1996).

Several studies document differences in physiology of macrogynes and microgynes and in genetic and social structure of colonies containing each queen form (Lachaud et al., 1999; McInnes and Tschinkel, 1995; Rüppell and Heinze, 1999; Rüppell et al., 2001a,b,c), but few such studies have been conducted on species that have winged and wingless queens (Buschinger and Schreiber, 2002; Fersch et al., 2000; Murakami et al., 2000; Yamauchi et al., 1991). Most notably, determination of winged versus wingless queens has a simple genetic basis in *Leptothorax* sp. A, *Harpagoxenus sublaevis*, and *Myrmecina graminicola* (Buschinger, 2005; Heinze and Buschinger, 1989; Winter and Buschinger, 1986).

The ant genus *Pogonomyrmex* has potential to serve as a model for understanding the evolution of wing development. The genus is moderate in size (~65 species), yet unrelated species produce both winged and wingless queens, unrelated species have brachypterous queens, and several species are thus far known to have only intermorph or ergatoid queens (Buschinger and Heinze, 1992; Heinze et al., 1992; Kusnezov, 1951). Additionally, workers of several species occasionally display sclerotization at the site where the wing would be inserted, similar to that displayed by some intermorph queens (R.A. Johnson, pers. obs.). Despite such variation in wing development, the only study on queen forms in this genus involves occurrence of intermorph and dealate queens in *P.* (*Ephebomyrmex*) *imberbiculus*, where information on queen dimorphism was limited to a population that contained only intermorph queens (dealate queens were extremely rare) (Heinze et al., 1992). *Pogonomyrmex imberbiculus* was inferred to be polygynous because colonies contained multiple inseminated, egg-laying intermorph queens. Heinze et al. (1992) also compared morphology of the three female castes of *P. imberbiculus*, but thorough analysis was hindered by lack of alate/dealate queens. Further understanding the dynamics of this queen dimorphism requires studying populations that contain both queen phenotypes.

This study was motivated by observations that winged (alate, or dealate after the wings are shed) and wingless (intermorph) queens co-occur in *Pogonomyrmex* (*Ephebomyrmex*) *pima* Wheeler (subfamily Myrmicinae) (see Heinze et al., 1992), and that colonies often contain multiple queens. This study extends our understanding of queen dimorphism for ants in general and this species in particular by: (1) conducting a morphometric analysis of workers, intermorph queens, and alate queens, (2) determining if alate queens and intermorph queens belong to the same gene pool, (3) determining the number and type of queens within colonies and their reproductive status, and (4) assessing for type of reproductive queens produced in colonies. Established colonies sometimes have queens that forage and participate in nest maintenance activities, and we also examined this behavior.

## Methods

### *Study site*

We studied *P. pima* at a site approximately 2 km south of the Sacaton Mountains, Pinal County, Arizona (32°56'N, 111°42'W; elevation 430 m). Habitat at the site was typical Sonoran Desert and consisted of a creosote bush (*Larrea tridentata*) association with scattered ironwoods (*Olneya tesota*). Substrate consisted of moderately coarse sand (see Johnson, 1992). Other common ants at the site included *Messor pergandei*, *Pheidole xerophila*, *Dorymyrmex insanus*, and *Solenopsis xyloni.*

### *Nest excavations*

We excavated nests of *P. pima* during April-May 2001–2005 to determine number of workers and the number and type of queens. Excavation of most nests involved digging a 3–4 cm deep depression that was about 15–20 cm in diameter centered on the nest entrance. We then dripped 1–1.5 liters of water onto the nest, which facilitated movement of individuals toward the surface; the colony was excavated 24–48 h later. A minority of nests was excavated without adding water. Rarely did either method result in a complete colony excavation on the first day. Thus, we revisited nests on subsequent days, and excavated colonies around the location in which workers had reopened tunnels to the surface; excavation of each nest continued until workers or evidence of their activities had not been observed for several days. Additional colonies were excavated during August-September (when unmated adult reproductives occur in nests) to assess the type of queens produced; these colonies were only excavated over one day. We collected all individuals from each nest and returned them to the laboratory, where each individual was examined under a binocular microscope and identified as worker, intermorph queen, or dealate queen.

### *Morphometric comparison of the three female castes*

We measured 10 morphological characters (eight external, two internal) for workers, intermorph queens, and alate queens of *P. pima.* External characters were head width, head length, scape length, maximum eye diameter, diameter of the anterior ocellus, pronotal width, petiole width, and post-petiole width; internal characters were number of ovarioles and presence/absence of a spermatheca (see also Heinze et al., 1992). The eight external characters were measured by projecting a 40x image from a binocular microscope to a video monitor; the image on the monitor was measured to 0.01 mm using NIH Image (available at http://rsb.info.nih.gov/nih-image/). The two internal characters were determined via dissections under a binocular microscope. Characters were measured on a maximum of two individuals per caste per colony.

We performed a morphometric analysis of the three female castes using multivariate analysis-of-variance (MANOVA). The data set included the seven continuous external characters; ocelli diameter was excluded from this analysis because workers lack ocelli (see below). An *a posteriori* univariate F test was used to determine which variables contributed to overall differences among the castes (SPSS, 1990).

Variables were transformed, as necessary, to meet the assumptions of MANOVA. We then assessed degree of overlap among the three castes by performing a discriminant analysis using these seven characters. The discriminant analysis developed predictive discriminant functions for each caste, which were then applied to all individuals during the same execution of the model (SPSS, 1990). The seven characters were entered into the model simultaneously using caste as the grouping variable. The model used *a priori* classification and equal prior probabilities.

The three discrete characters (diameter of the anterior ocellus, ovariole number, and presence/absence of a spermatheca) were compared across the three castes using a Kruskal-Wallis test (Siegel and Castellan, 1988).

### *Lineage analysis of alate and intermorph queens*

We assessed whether alate queens and intermorph queens belong to the same gene pool using microsatellite allele frequencies and mitochondrial gene sequences. DNA was extracted using a standard Chelex protocol in which we added 50 μl of 5% Chelex and 25 mg Protease K to individuals that had been preserved in 95% ethanol, then dried and crushed in liquid nitrogen. Samples were then incubated at 57°C for 1 h, boiled at 95°C for 5 min, and centrifuged at 14,000 rpm for 10 min. Supernatant was removed and stored at -20°C.

We compared microsatellite allele frequencies of alate and intermorph queens using four polymorphic loci: (1) L18 (Foitzik et al., 1997), (2) LxAGT1 (Bourke et al., 1997), (3) Myrt3 (Evans, 1995), and (4) Pb8 (Volny and Gordon, 2002). PCR's were performed in reaction volumes of 25 μl: 1.0 μl diluted (1:10) template DNA, 2.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.4 μM of each primer (labeled with IRDye 700 or IRDye 800; LI-COR), 5.0 μl of 5x *GoTaq* buffer, and 0.5 U *Taq* DNA polymerase (Promega). We used the following PCR touchdown profile: denaturation for 5 min at 94°C, followed by 15 cycles of 45 s at 94°C, 45 s at 60°C-45°C (-1°C per cycle), and 45 s at 72°C, then 20 cycles of 45 s at 94°C, 45 s at 55°C (L18: 53°C), and 45 s at 72°C, followed by an elongation step of 10 min at 72°C.

PCR products were genotyped using a LI-COR 4300 DNA analyzer. Alleles were homologized by running individuals from all colonies on one gel, then scored using the program SAGA (LI-COR). We genotyped 7 alate queens and 9 intermorph queens (one per colony), and we deduced genotypes of 11 additional queens (7 alates and 4 intermorphs) from offspring genotypes using MateSoft 1.0 (Moilanen et al., 2004).

Based on these queen genotypes, we calculated heterozygosity and F-statistics, and conducted Chi-square tests for Hardy-Weinberg and linkage equilibrium using the program GDA 1.1 (Lewis and Zaykin, 2002). We tested for nuclear genetic subdivision between alate and intermorph queens using a Bayesian model-based clustering algorithm implemented in Structure 2.1 (Pritchard et al., 2000). The latter procedure assigns individuals to $K$ clusters without prior assumptions of population origin. We ran the admixture model, the no admixture model, and the correlated allele frequencies model at $K = 1$ and $K = 2$, with each run consisting of 100,000 iterations after a burn-in period of 100,000. We conducted five runs per $K$ to verify estimates; the mean log likelihood value was used to compute probability for each $K$. We also calculated the $F_{ST}$ value between alate and intermorph queens.

For mitochondrial sequences, we used the LCO and HCO primers to amplify a 656 bp region of the cytochrome c oxidase subunit 1 (CO1) for one queen or worker from 15 dealate and 13 intermorph queen colonies (Folmer et al., 1994). We rooted our phylogeny by sequencing one worker each of *P. pima* from Mohawk Dunes, Yuma County, AZ (~200 km W of our study site), *P.* (*E.*) *imberbiculus* (GenBank accession #*AY510614*), *P. (E.) naegalii* (Argentina: Entre Rios Province; RAJ #3631), and *P. (P.) barbatus* (AZ: Pinal County; RAJ #3896). PCR's were performed, as above, with an initial denaturation of 4 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 45°C, and 1 min at 68°C, then elongation of 4 min at 68°C. We added 2.5 U Exonuclease 1, 2.5 U Shrimp Alkaline Phosphatase, and 0.5 μl dilution buffer (USB) to 6 μl of each amplicon, then incubated samples for 15 min at 37°C, and for 15 min at 80°C. Purified products were sequenced in both directions using an Applied Biosystems 3730 DNA analyzer, then aligned using BioEdit 7.0.5.3 (Hall, 1999).

Phylogenetic relationships were inferred using the neighbor-joining (Kimura 2-parameter) and maximum parsimony (close-neighbor-interchange with random addition) programs in MEGA 3.1 (Kumar et al., 2004). Branch support was calculated from 1,000 bootstrap replicates. Within-morph and between-morph differences in sequence divergence were compared by pairwise Mann-Whitney $U$ tests (Mueller and Ayala, 1982). Sequences are deposited in GenBank (accession #'s EF044144-EF044172). Voucher specimens were deposited at MCZ (Harvard University, USA), the collections of Robert A. Johnson (Tempe, Arizona, USA) and Philip S. Ward (University of California, Davis, USA), and Museum Koenig (Bonn, Germany).

### *Dissection of intermorph and dealate queens*

We determined number of inseminated queens in colonies by dissecting out the reproductive tract of individuals under a binocular microscope ($n = 125$ dealate queens from 9 colonies; $n = 126$ intermorph queens from 10 colonies). Color of the spermatheca was used to assess mating status (inseminated or uninseminated): queens were scored as unmated if their spermatheca was clear and translucent, whereas those with an opaque or whitish spermatheca were scored as mated (see Alloway et al., 1982; Keller and Passera, 1993). We verified this assessment by examining the spermatheca of virgin alate queens and newly mated dealate queens.

### *Foraging by queens in established colonies*

We observed queen foraging on several occasions, and in each case the queen returned to an established colony. Such queen foraging is unusual among ants, and little is known about the behavior. Consequently, we collected data on two aspects of queen foraging: (1) the proportion of colony foraging trips made by queens, and (2) reproductive status (mated or unmated) of foraging queens. Relative foraging effort was quantified by observing two colonies that had foraging queens. For each colony, we recorded status (worker or queen) of 25–30 successive foragers returning to the nest on each of two days. We determined mating status of foraging queens by dissecting out the reproductive tract of individuals ($n = 20$) under a binocular microscope (see above). We also examined the ovarioles for developing oocytes or for evidence that they had become expanded for reproduction.

## Results

### *Morphometric comparison of the three female castes*

Size of the three female castes of *P. pima* differed significantly (Wilks' $\lambda = 0.105$, $F_{14,102} = 15.2$, $P << 0.0001$); all seven continuous characters varied among the three castes (univariate $F$ tests within MANOVA, $P < 0.001$), as did all three discrete characters (Table 1). The pattern of variation differed by character; measures for intermorph queens were intermediate to those of workers and alate queens for five characters, similar to those of alate queens for four characters, and similar to those of workers for one character (Duncan's multiple range test for continuous characters, Kruskal-Wallis test for discrete characters, $P < 0.05$; Table 1).

Discriminant analysis correctly classified 91.7% (55 of 60) of all individuals (Table 2). Misclassification of individuals involved only workers and intermorph queens; two workers were classified as intermorph

**Table 1.** Morphological measures (mean ± 1 SE) for the three female castes of the seed-harvester ant *Pogonomyrmex* (*Ephebomyrmex*) *pima* (in mm). $N = 20$ individuals per caste ($\leq 2$ individuals per colony). Significant differences among castes are given by the letters $a–c$: $a > b > c$. Groupings are based on univariate F tests within MANOVA followed by a Duncan's multiple range test for continuous variables or a Kruskal-Wallis test for discrete characters (see text). Characters in **bold font** were used in the discriminant analysis.

| Character* | Worker | Caste | |
|---|---|---|---|
| | | Intermorph queen | Alate queen |
| Head | | | |
| **Head width (HW)** | 1.05 ± 0.01$^{b}$ | 1.11 ± 0.01$^{a}$ | 1.10 ± 0.01$^{a}$ |
| **Head length (HL)** | 1.09 ± 0.01$^{b}$ | 1.14 ± 0.01$^{a}$ | 1.15 ± 0.01$^{a}$ |
| **Maximum ocular diameter (MOD)** | 0.24 ± 0.00$^{c}$ | 0.26 ± 0.00$^{b}$ | 0.28 ± 0.00$^{a}$ |
| **Scape length (SL)** | 0.73 ± 0.01$^{b}$ | 0.75 ± 0.01$^{b}$ | 0.77 ± 0.01$^{a}$ |
| Ocellus diameter (OD) | Absent$^{c}$ | 0.03 ± 0.00$^{b}$ | 0.06 ± 0.00$^{a}$ |
| Mesosoma | | | |
| **Pronotal width (PW1)** | 0.72 ± 0.01$^{c}$ | 0.80 ± 0.01$^{b}$ | 0.85 ± 0.01$^{a}$ |
| **Petiole width (PW2)** | 0.29 ± 0.00$^{c}$ | 0.32 ± 0.01$^{b}$ | 0.35 +0.00$^{a}$ |
| **Post-petiole width (PW3)** | 0.44 ± 0.01$^{c}$ | 0.52 ± 0.01$^{b}$ | 0.54 ± 0.01$^{a}$ |
| Gaster | | | |
| Number of ovarioles | 2.00 ± 0.00$^{b}$ | 7.86 ± 0.14$^{a}$ | 7.55 ± 0.17$^{a}$ |
| Spermatheca present | No | Yes | Yes |

* HW – maximum width of the head, positioned in full face view, at a level above the upper eye margin; HL – Maximum length of the head, positioned in full face view, from the midpoint of the anterior clypeal margin to the midpoint of the occipital margin; MOD – maximum diameter of the eye as measured with the head in full lateral aspect; SL – maximum length of the scape, excluding the basal condyle; OD – maximum diameter of the anterior ocellus; PW1–3 – maximum width of pronotum, petiole, and post-petiole, respectively, as seen from above, at right angles to the longitudinal axis of the mesosoma.

**Table 2.** Summary classification of predicted group membership for workers, intermorph queens, and alate queens of *Pogonomyrmex* (*Ephebomyrmex*) *pima* using discriminant analysis. The seven morphological characters used in the analysis are given in Table 3. Values are the number of individuals that discriminant analysis classified into each of the three female castes.

| Caste | Predicted group membership | | | |
|---|---|---|---|---|
| | Worker | Intermorph queen | Alate queen | Total correct |
| Worker | 18 | 2 | 0 | 18/20 (90.0) |
| Intermorph queen | 2 | 17 | 1 | 17/20 (85.0) |
| Alate queen | 0 | 0 | 20 | 20/20 (100.0) |
| Total | 20 | 19 | 21 | 55/60 (91.7) |

queens, and several intermorph queens were classified as workers or alate queens (Table 2, Fig. 1). As might be expected from the MANOVA, intermorph queens occupied a multivariate space intermediate to that of workers and alate queens. This intermediacy was also demonstrated by group centroids, with the centroid for intermorph queens being nearly equidistant to those of workers and alate queens (Fig. 1). The standardized coefficients of the canonical functions indicated that head width and pronotal width were the primary contributors to discriminant function 1, which accounted for 82.1 % of the variance. Width of the petiole and post-petiole were the primary contributors to discriminant function 2, which accounted for the remaining 17.9 % of the variance (Table 3).

**Table 3.** Standardized coefficients for canonical discriminant functions for workers, intermorph queens, and alate queens of *Pogonomyrmex* (*Ephebomyrmex*) *pima*.

| Predictor variable | Function 1 | Function 2 |
|---|---|---|
| Head width | – 1.045 | 0.709 |
| Head length | – 0.101 | 0.444 |
| Maximum ocular diameter | 0.526 | – 0.365 |
| Scape length | – 0.200 | 0.110 |
| Pronotal width | 1.027 | – 0.624 |
| Petiole width | 0.244 | – 1.107 |
| Post-petiole width | 0.165 | 1.502 |

Intermorph queens were intermediate in size to workers and alate queens, but their overall morphology and specialized reproductive structures were most similar to those of alate queens. Both intermorph and alate queens possessed a spermatheca (which all workers

**Table 4.** Genetic variation of *Pogonomyrmex* (*Ephebomyrmex*) *pima* at the four microsatellite loci used in this study. $H_e$ = expected heterozyosity; $H_o$ = observed heterozygosity; $F_{IS}$ = inbreeding coefficient. $N$ = 27 queens (1 per colony).

| Locus | # of alleles | Size range (bp) | $H_e$ | $H_o$ | $F_{IS}$ |
|---|---|---|---|---|---|
| L18 | 2 | 131–136 | 0.409 | 0.407 | 0.003 |
| LxAGT1 | 23 | 212–274 | 0.947 | 0.815 | 0.142 |
| Myrt3 | 7 | 166–186 | 0.711 | 0.815 | –0.149 |
| Pb8 | 13 | 273–297 | 0.817 | 0.769 | 0.059 |

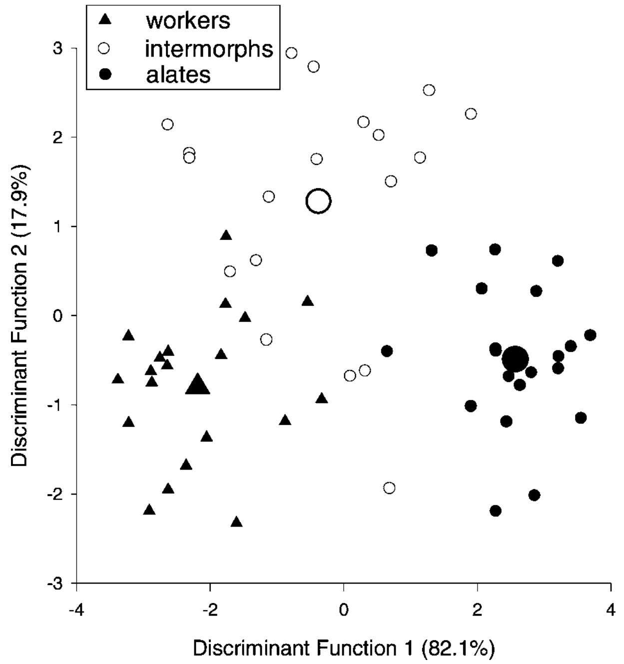

**Figure 1.** Discriminant scores for the three female castes of the ant *Pogonomyrmex* (*Ephebomyrmex*) *pima*. Scores of all individuals ($n$ = 20 per caste) are projected onto the two-dimensional space defined by discriminant functions one and two. The larger symbols for each caste are the unstandardized canonical discriminant functions evaluated at the group means (centroids).

lacked), had a similar number of ovarioles (Table 1), and laid eggs that developed into workers. Additional anatomical structures common to both types of queens included ocelli (which workers lacked) and an enlarged and dorsally flattened mesosoma with a distinct suture between the sclerites; the mesosoma of workers was reduced and rounded, and the sclerites were completely fused (Fig. 2). Intermorph and alate queens also have coarse longitudinal rugae on the dorsum of the mesosoma, which contrasts with the coarse rugo-reticulose sculpturing on the mesosoma of workers. Note, however, that intermorph queens varied in degree of expression of some queen-like structures. For example, the site where the wing would be inserted was marked by a small, darkly pigmented spot on some intermorphs, while this spot was lacking on other intermorphs (Heinze et al., 1992; Heinze and Tsuji, 1995).

### *Lineage analysis of alate and intermorph queens*

Microsatellite data and the mitochondrial sequence phylogeny indicated that alate and intermorph queens belong to the same gene pool. Microsatellite allele frequencies conformed to those expected under Hardy-Weinberg equilibrium ($P > 0.05$ for all four loci), and there was no evidence of linkage disequilibrium between loci ($P > 0.05$). The 95 % confidence interval (CI) of $F_{IS}$ bootstrapped across loci (-0.093–0.123) overlapped with zero, indicating an absence of inbreeding. The $F_{ST}$ value of 0.027 (95 % CI: 0.009–0.052) was significantly different from zero, suggesting very weak population subdivision between alate and intermorph queens. Alternatively, cluster analysis of the microsatellite data indicated a lack of genetic differentiation between alate and intermorph queens because the $K = 1$ model had a much higher probability (0.98) than the $K = 2$ model (0.02). We obtained similar results running models with no admixture and correlated allele frequencies (results not shown).

The neighbor-joining tree (Fig. 3) and the maximum parsimony tree (not shown) yielded similar topologies for our mitochondrial sequences. Intermixing of alate and intermorph queens throughout both trees demonstrated that the two phenotypes belong to the same mitochondrial clade (Fig. 3). This conclusion is also supported by an analysis showing that sequence divergence values between the two queen phenotypes (mean pairwise distance ± SE = 0.019 ± 0.002) did not differ from pairwise distances within each phenotype (alates: 0.013 ± 0.002, $U = 8174.5$, $P = 0.104$; intermorphs: 0.029 ± 0.004, $U = 8289.5$, $P = 0.244$). In fact, some intermorph and alate/dealate queens had identical mitochondrial haplotypes.

### *Colony structure and size*

Multiple queens occurred in 43 of the 60 (71.7 %) *P. pima* colonies in which we found queens. Occurrence of dealate and intermorph queens in these colonies was highly nonrandom because 42 of the 43 colonies (97.7 %) contained only one type of queen ($n = 216$ dealate queens in 16 colonies; $n = 296$ intermorph queens in 26 colonies); the one exceptional colony contained five intermorph queens and one dealate queen. At the population level, a similar number of colonies contained only dealate ($n = 23$) or only intermorph queens ($n = 36$) ($\chi^2 = 2.9$, 1 *df*, $P > 0.05$). Number of queens per colony was similar for both types of queens (dealate queens: mean = 9.7 ± 2.6, intermorph queens: mean = 8.5 ± 1.9: t-test, t = 0.4, 57 *df*, $P > 0.50$).

Dissections indicated that colony structure differed for dealate and intermorph queen colonies. Only 1 of 125 dealate queens (0.8 %; $n = 9$ colonies) was inseminated, compared to 43 of 126 intermorph queens (34.1 %; $n = 10$ colonies). All inseminated queens had expanded ovarioles and developing oocytes, suggesting that they were functionally fertile. Moreover, eight of 10 intermorph

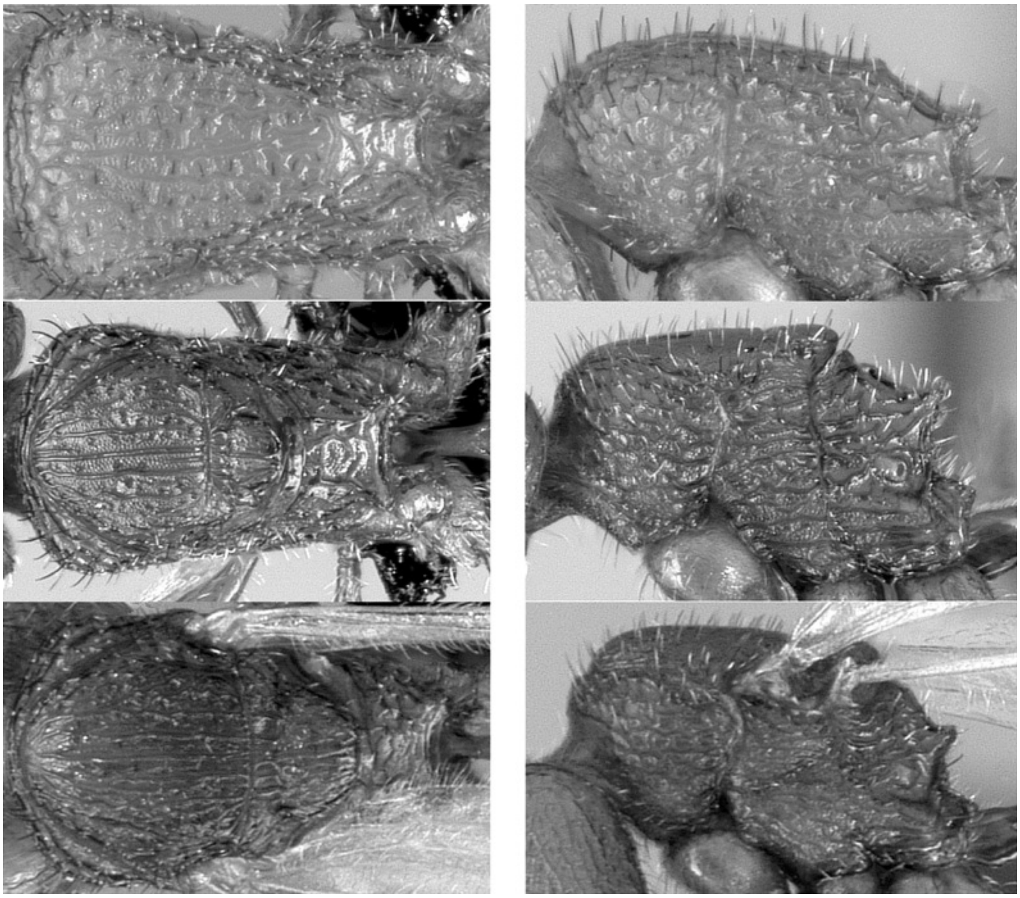

**Figure 2.** Dorsal (left) and profile (right) view of the mesosoma for a worker (top), intermorph queen (center), and alate queen (bottom) of the ant *Pogonomyrmex* (*Ephebomyrmex*) *pima.* Color photographs are available at http://asu.edu/clas/sirgtools/pogonomyrmex/NORTHAMERICANPOGOS.htm.

queen colonies were functionally polygynous (range = 2–14 mated queens per colony), whereas we rarely found a mated queen in dealate queen colonies. We attribute this latter result to the fact that these data came from partially excavated colonies.

All colonies excavated during the reproductive season contained only one queen phenotype. Of 13 colonies with a dealate queen, seven colonies contained alate females and males, four colonies contained only alate females, and two colonies contained only males (males: mean = 17.0, range 0–65; alate females: mean = 37.3, range = 0–105). In addition, one laboratory colony headed by a dealate queen produced several alate females. Of the 12 intermorph queen colonies, 10 colonies contained intermorph queens and males, and two colonies contained only intermorph queens (males: mean = 48.9, range 0–261; intermorph queens: mean = 13.5, range = 1–57).

Colony size (number of workers) ranged from 52–326 for the five colonies that we judged to have been completely excavated during April-May. However, the largest colony, which was excavated in late September, contained 449 workers and one intermorph queen. Data from partially excavated colonies indicated that they typically contain >100 workers. These limited data suggest that the maximum number of workers (April-May data only) is similar for colonies regardless of the queen phenotype (intermorph queen: $N_{max} = 309$ workers; dealate queen: $N_{max} = 326$ workers).

### *Queen foraging*

Foraging queens accounted for a variable but sometimes substantial proportion of the foraging trips for colonies in which they occurred (mean = 21.7 ± 6.5%, range = 3.3–33.3%, $n = 116$ foraging trips collected over two observation periods for each of two colonies). Examination of the spermatheca indicated that all 20 foraging queens (2 intermorph, 18 dealate) were unmated. Surprisingly, 8 of the 20 queens contained one or more ovarioles that had become expanded and contained a large white vitellogenized oocyte; ovarioles in the other 12 queens were translucent and undeveloped, similar to those of alate queens. Presence of expanded ovarioles suggested that unmated queens can retain reproductive capability. We investigated this possibility by placing several dealate queen foragers plus workers from one colony into a small plaster-lined nest box inside a darkened incubator at 35° C. The plaster was moistened periodically and queens were provided fruit flies every 1–3 days. These queens laid numerous eggs, and the several adults that emerged were all males. We doubt that

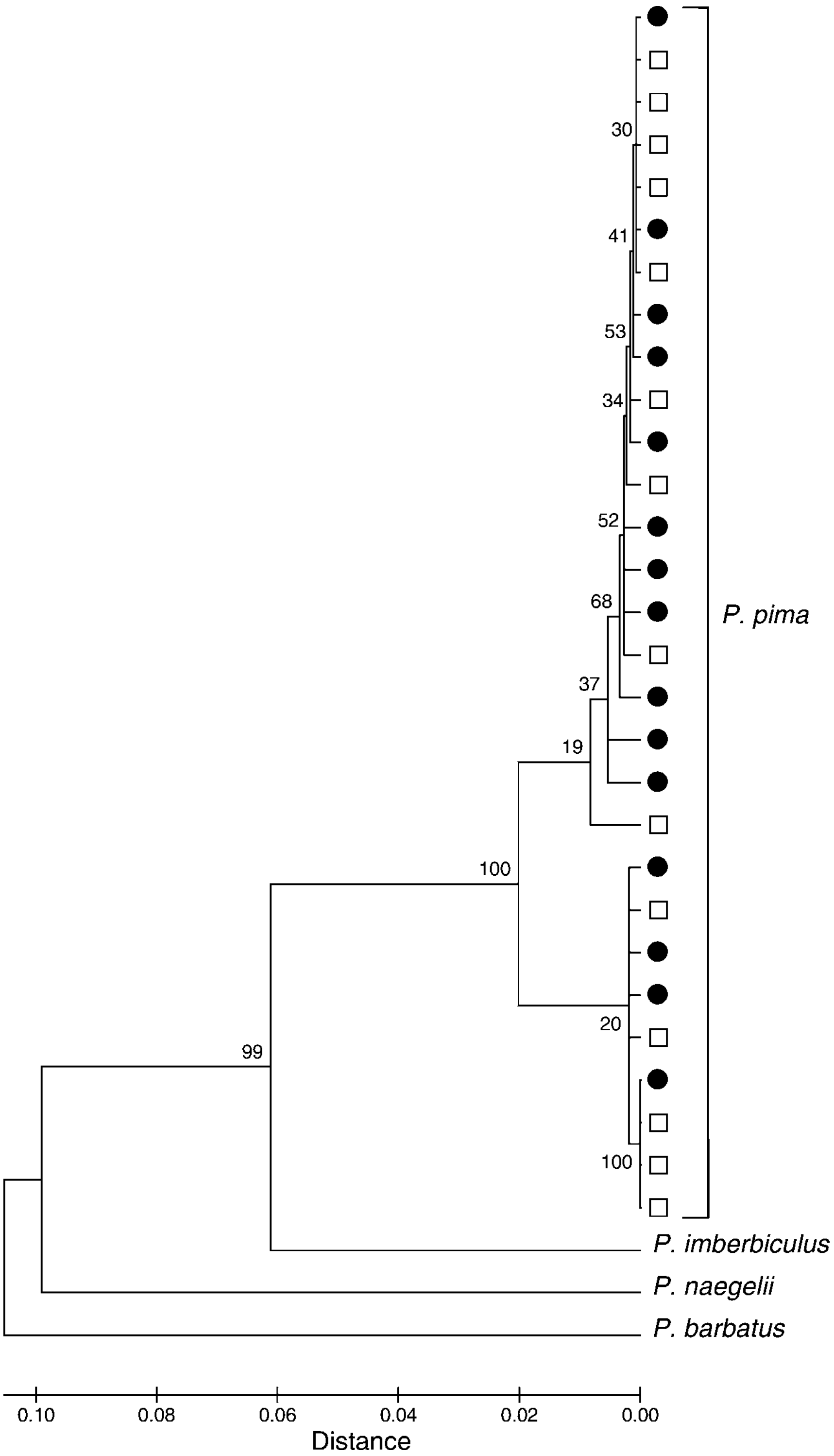

**Figure 3.** Neighbor-joining tree for alate and intermorph queens of the ant *Pogonomyrmex* (*Ephebomyrmex*) *pima* from our study site in Pinal County, Arizona; ● = alate queen; ○ = intermorph queen. The tree was constructed using Kimura 2-parameter pairwise distances between sequences for a 656 bp region of the CO1 mitochondrial gene; 73 sites were parsimony informative. Bootstrap values (1,000 repetitions) are given at branch nodes. See text for information on outgroup samples.

workers produced any of these males for two reasons: (1) all workers that we dissected lacked enlarged ovarioles and oocytes, which characterize egg-laying, and (2) we did not observe eggs in any queenless colonies.

## Discussion

### Evolution of queen dimorphism

Dispersal polymorphisms result from the ability of organisms to produce multiple phenotypes, i.e., polyphenisms. Polyphenisms occur in a wide variety of insects, and they are particularly well known in crickets, aphids, and ants (Zera, 2004). Alternate phenotypes can be triggered by genetic factors, environmental factors, or a combination of both types of factors (Nijhout, 1999; Zera, 2004). Our microsatellite data demonstrated unrestricted gene flow between the two queen forms, along with an absence of assortative mating and inbreeding by males. Consequently, it is doubtful that a single diallelic locus (or several loci) causes the polyphenism in *P. pima* (Heinze and Buschinger, 1989; Roff, 1986; Winter and Buschinger, 1986). Such a mechanism would result in some intermorph queens producing both winged and wingless daughters, whereas we found that colonies contained only one queen phenotype. Consequently, it seems likely that this polyphenism results from an environmental factor, maternal effects, or from direct or indirect pheromonal effects of the queen.

Intraspecific queen dimorphisms in the form of winged and wingless phenotypes are uncommon in ants, with examples scattered across genera in several subfamilies (Heinze and Tsuji, 1995). In the genus *Pogonomyrmex*, a queen wing dimorphism occurs in at least four species, the two North American sister species *P.* (*E.*) *imberbiculus* and *P.* (*E.*) *pima* (this study, Heinze et al., 1992), and the two South American species *P.* (*E.*) *naegalii* and *P.* (*P.*) *uruguayensis* (R.A. Johnson, unpubl. data). Additionally, ergatoids or intermorphs are the only queen form thus far known for several other species in the subgenera *Pogonomyrmex* and *Ephebomyrmex*, as well as for the only species in the subgenus *Forelomyrmex* (Kugler, 1978; Kugler and Hincapie, 1983; Kusnezov, 1951; R.A. Johnson, unpubl. data). The widespread geographic and taxonomic occurrence of intermorph/ergatoid queens suggests that (1) wingless queens represent an ancestral trait in the genus, or (2) wingless queens have evolved multiple times, possibly as a secondary modification in response to local ecological conditions (Heinze and Tsuji, 1995; Zera and Denno, 1997). Wingless queens appear to be somewhat common in desert habitats (Heinze and Tsuji, 1995; Tinaut and Heinze, 1992), which may explain the occurrence of intermorph queens in *P. pima*, whose distribution is restricted to the Sonoran Desert. A first step toward understanding factors that drive the evolution of queen wing phenotypes in *Pogonomyrmex* involves collecting information on queen forms for additional species, followed by mapping this trait onto a phylogeny that contains species from North America and South America.

### *Alternative life histories*

Queen dimorphisms often result in alternative life histories that include differences in colony social structure and mode of dispersal (Rüppell and Heinze, 1999; Rüppell et al., 2001a,c). The typical pattern for colony social structure is that intermorph queen colonies are polygynous, whereas dealate queen colonies are monogynous (Buschinger and Schreiber, 2002; Fersch et al., 2000). Our dissection data suggest that *P. pima* parallels this pattern given that multiple inseminated intermorph queens occurred in eight of 10 colonies, as was also found in *P. imberbiculus* (Heinze et al., 1992).

In contrast, lack of mated dealate queens in all but one colony suggests that these colonies are monogynous, but additional data are needed to substantiate this result. Regardless, colony structure appears to differ for dealate and intermorph queen colonies given that we dissected a similar number of each phenotype. Our inability to find mated dealate queens in these colonies probably resulted from difficulty in excavating the entire colony and reproductive queen, especially given that these colonies were only excavated over one day. Lastly, our few complete colony excavations indicate that colony size (number of workers) was not affected by type or number of queens.

Mode of dispersal and method of colony founding also typically differ for the two queen phenotypes (Hölldobler and Bartz, 1985; Peeters and Ito, 2001). In *P. pima*, alate queens fly to a mating aggregation, then found colonies haplometrotically (R.A. Johnson, unpubl. data). Alternatively, observations indicate that intermorph queens return to their natal colony after mating. Intermorph queens have also been observed at mating aggregations, suggesting that some of these queens might found colonies independently. This possibility is supported by laboratory experiments demonstrating that single intermorph queens can produce several workers if they are provided food (R.A. Johnson, unpubl. data). Moreover, intermorph and dealate queens are similar in that both queen forms must forage to obtain sufficient resources to rear their first workers, i.e., they are obligate foragers (Johnson, 2006; Schrempf and Heinze, 2006).

### *Morphometrics*

Intermorph queens are considered to be a permanent evolutionary modification of the ancestral queen morphology that constitute an integral part of the life history of a species (Heinze, 1998). In *P. pima*, intermorph queens have reproductive ability similar to that of alate queens, and they also display anatomical and reproductive features that characterize alate queens, except for their loss of wings. This study provides one of the first multivariate studies of intermorph queens (see also Murakami et al., 2002; Visicchio et al., 2003), and shows that intermorph queens are intermediate in size between that of workers and alate queens (Fig. 1); this pattern also occurs for intermorph queens of other species (Heinze et al., 1992; Murakami et al., 2002). Interestingly, size of individual characters varied for intermorph and alate queens. Head size (length and width) was similar for intermorph and alate queens, but the mesosoma (pronotal width), petiole, and post-petiole were consistently smaller in intermorph queens. The smaller mesosoma of intermorph queens likely reflects loss of wings and associated musculature.

### *Queen foraging*

Several ant species have winged queens that sometimes forego mating and later perform tasks such as foraging and nest maintenance (Brown, 1999; Forder and Marsh, 1986; Peeters, 1997). Both dealate queens and intermorph queens of *P. pima* also perform such tasks, and queens that engage in such behaviors are invariably unmated. Few data exist on species in which unmated queens opt to forage, but observations suggest that foraging queens are short-lived in *Messor andrei* and *Acromyrmex versicolor* (Brown, 1999; J. Weser, pers. comm.). In contrast, foraging queens of *P. pima* appear to be longer-lived given their occurrence in colonies during May, about eight months after the sexual brood are normally produced. Foraging queens can represent a variable but sometimes significant portion of the foraging trips made by colonies, especially given that 10 or more foraging queens can occur in a colony.

The mechanism that causes queens to forego mating and to perform worker-like tasks is unknown. One possibility may relate to occurrence of the rains that trigger their mating flights. Mating flights for both *P. pima* and *A. versicolor* often depend on late season monsoon rains, which do not occur in some years. In such years, queens might make the best of a bad situation by dealating themselves, then performing worker-like behaviors that can benefit the colony. However, this mechanism does not explain foraging by unmated queens of *M. andrei* given that their mating flights are not triggered by rainfall (Johnson, 2000). Regardless of the mechanism causing this behavior, it appears that some foraging queens of *P. pima* can produce males. Hence, staying in the natal colony may allow them some opportunity to reproduce.

Overall, this study documents that *P. pima* is one of few ant species that exhibits an intraspecific queen dimorphism in which winged queens are common, and in which colonies produce only one queen phenotype (Heinze and Tsuji, 1995). Our data suggest that *P. pima* is ideal for examining both proximate and ultimate causes of caste determination. At the proximate level, this system provides opportunities to examine mechanisms that trigger caste determination as well as those that determine body size and wing development. At the ultimate level, determining the phylogenetic occurrence of intermorph/ergatoid queens across the genus would provide insight into the evolution of alternative queen phenotypes.

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