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Dynamics of an ant–ant obligate mutualism: colony growth, density dependence and frequency dependence

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Abstract

In insect societies, worker vs. queen development (reproductive caste) is typically governed by environmental factors, but many *Pogonomyrmex* seed-harvester ants exhibit strict genetic caste determination, resulting in an obligate mutualism between two reproductively isolated lineages. Same-lineage matings produce fertile queens while alternate-lineage matings produce sterile workers. Because new virgin queens mate randomly with multiple males of each lineage type, and both worker and queen phenotypes are required for colony growth and future reproduction, fitness is influenced by the relative frequency of each lineage involved in the mutualistic breeding system. While models based solely on frequency-dependent selection predict the convergence of lineage frequencies towards equal (0.5/0.5), we surveyed the lineage ratios of 49 systems across the range of the mutualism and found that the global lineage frequency differed significantly from equal. Multiple regression analysis of our system survey data revealed that the density and relative frequency of one lineage decreases at lower elevations, while the frequency of the alternate lineage increases with total colony density. While the production of the first worker cohort is largely frequency dependent, relying on the random acquisition of worker-biased sperm stores, subsequent colony growth is independent of lineage frequency. We provide a simulation model showing that a net ecological advantage held by one lineage can lead to the maintenance of stable but asymmetric lineage frequencies. Collectively, these findings suggest that a combination of frequency-dependent and frequency-independent mechanisms can generate many different localized and independently evolving system equilibria.

Keywords: co-evolution, dependent lineage, frequency-dependent selection, obligate mutualism, polyphenism, symmetrical social hybridogenesis

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Introduction

Obligate mutualisms involve pairs of reproductively isolated genomes that require one another for survival or reproduction. The stability of mutualisms and other types of co-evolving systems across space and time depends on variation in biotic and environmental factors, as well as variation within each co-evolving population (Thompson 1999; Gomulkiewicz *et al.* 2000, 2003; Nuismer *et al.* 2003). Geographical variation in such factors can lead to a mosaic pattern of co-evolution wherein the same interspecific interaction varies across the landscape (Travis 1996; Thompson 1999). In this contribution, we explore an ant–ant obligate mutualism across a broad geographical range. We are primarily interested in two selection mechanisms: frequency-dependent selection and density-dependent selection. In an obligate mutualism, frequency-dependent selection occurring between species acts to maintain the relative numbers of each species necessary for the interaction (Holland & DeAngelis 2001). However, this balancing act can be affected by environmental heterogeneity resulting from density-dependent selection or the

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degree of adaptation of one or the other species to the local environment.

Some *Pogonomyrmex* harvester ants are locked in an obligate mutualism wherein two reproductively isolated lineages depend on one another to provide the genetic components needed to produce workers (for a review see Anderson *et al.* 2008). As with all mutualisms, the genomes of each lineage must co-evolve, in this case to retain the reciprocal phenotypes required to produce workers and to track many components of the same external environment (Anderson *et al.* 2008). The mechanism that underlies this ant–ant obligate mutualism is reproductive caste determination; the process whereby individual female larvae develop as new virgin queens (gynes) or sterile worker phenotypes. In the social Hymenoptera, reproductive caste is typically determined by environmental factors such as nutrition, and caste expression is considered a type of polyphenism because diploid eggs of the same genotype may be raised by the colony as either a sterile worker or a reproductive queen phenotype (Wheeler 1986). While the paradigm of environmental caste determination is supported by both theory and data (Hölldobler & Wilson 1990), research in a growing number of social insect systems has demonstrated that the reproductive caste in some social insects can be strongly influenced by genotype generating questions about the origin and maintenance of genetic variability for reproductive caste phenotypes (Anderson *et al.* 2008; Hölldobler & Wilson 2008; Hughes & Boomsma 2008; Smith *et al.* 2008; Schwander *et al.* 2010). The ability of the colony to control the reproductive caste is critical for colony efficiency because ant colonies rely on a robust worker caste for future reproduction. Analogous to long-lived plants that postpone flowering, ant colonies will delay the production of sexual offspring until the workforce surpasses a critical size threshold (Oster & Wilson 1978).

In mutualistic *Pogonomyrmex*, the reproductive/sterile caste distinction is determined strictly according to genotype (Genetic caste determination, Anderson *et al.* 2006a, 2008). Each localized group of interbreeding colonies or 'system' is composed of two morphologically cryptic and reproductively isolated lineages (Helms Cahan & Keller 2003; Anderson *et al.* 2006a; Helms Cahan *et al.* 2006; Schwander *et al.* 2006, 2007a; Curry *et al.* 2009). Each lineage lacks the genetic programming to generate the worker phenotype from within-lineage mating, so queens must acquire sperm from alternate-lineage males to produce workers (Helms Cahan *et al.* 2004). Thus, every colony in the system consists entirely of F_1 inter-lineage workers, but the gene pools of each lineage remain reproductively isolated because the workers are functionally sterile and next-generation reproductive females result from same-lineage matings. This obligate reproductive mutualism has been labelled a dependent lineage (DL) system (Anderson *et al.* 2006a; Fig. 1).

Maintenance of a DL system generates unique fitness consequences across many levels of selection. System persistence relies on obligate polyandry because new founding queens must mate with males of each lineage to later generate both gynes and workers (Helms Cahan *et al.* 2002; Julian *et al.* 2002; Volny & Gordon 2002). Queens mate randomly on a single occasion and all of the sperm for future colony fitness is stored in her spermatheca (Hölldobler 1976). Because males result from unfertilized eggs and are produced somewhat constitutively by reproductively mature colonies, the frequency of males of each lineage in the mating swarm is equivalent to the frequency of each lineage in the system (Anderson *et al.* 2009). Thus, polyandry and random mating result in queens of each lineage acquiring on average stored sperm ratios that reflect the relative proportions of each lineage in the system (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006).

Newly mated queens do not forage for food, but quickly dig a shallow nest and generate the first cohort of workers by metabolizing the nutritional reserves stored in her abdomen and flight muscles. Selection at this stage of the life cycle is severe owing to predation,

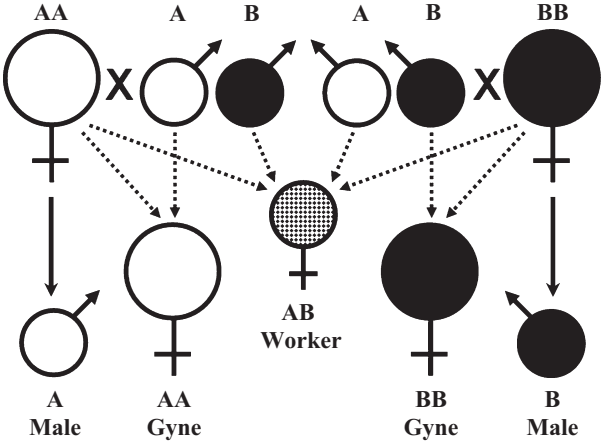

Fig. 1 Phenotypes and gamete types represented in a dependent lineage system. A and B are haploid males and AA, BB, and AB are diploid females. Females with AB genomes are genetically fated to develop as sterile workers, while AA and BB females develop as reproductive gynes (virgin queens). Dotted arrows show the diploid genomes resulting from haploid gametes and sexual reproduction, solid arrows represent haploid males produced via arrhenotokous parthenogenesis. To produce a colony capable of both worker and gyne production, the colony queen (AA or BB) must mate with both A and B males. Thus, the perpetuation of a DL system is dependent on the co-evolution and continued interaction of four types of gametes (Parker 2004).

stochastic factors and worker/gyne sperm ratios of the newly mated queen. On average, the queen will produce 2–5 small workers (called minims) in the first cohort, which must then compete for resources in an environment that is typically overdispersed with reproductively mature colonies containing many thousands of workers. Newly mated queens from a low-frequency (rare) lineage will on average have more alternate-lineage matings and possess proportionately more worker-destined sperm. Queens fertilize eggs randomly, and there is no sperm selection such that sperm ratios of rare-lineage queens translate directly into a higher proportion of worker-destined genotypes in the first cohort (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006). At the same time, common-lineage queens will on average acquire sperm ratios biased towards gyne production, in turn generating more gyne-destined genotypes among the first cohort. Gynes are 10× larger than minim workers and cannot complete their genetic programming owing to a lack of available nutrition. Thus, the initial colony founding success of queens of the different lineages is negatively frequency dependent (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006). Additionally, the final sex ratio of mature colonies is associated with relative lineage frequencies. While both lineages produce males in proportions that reflect relative lineage frequencies, increasing lineage rarity results in reduced gyne production by the rare lineage owing to the decreased availability of same-lineage sperm in the mating flight (Anderson *et al.* 2009). Thus, gyne production by reproductively capable colonies is positively frequency dependent. The opposing advantages of positive and negative frequency-dependent selection have been proposed as the basic mechanism for the persistence of the system (Yamauchi & Yamamura 2006).

As detailed earlier, the overall fitness costs for the system as a whole are expected to increase rapidly as the relative proportions of the two lineages become increasingly unequal. Deterministic models indicate that lineage frequencies and sex ratios may be subject to large fluctuations, suggesting that a DL system may persist only under a narrow set of parameter space (Yamauchi & Yamamura 2006; Helms Cahan & Julian 2010). Indeed, these models predict that the persistence of a DL system may only be possible with rapid convergence of each lineage towards equal frequencies (but see Anderson *et al.* 2009). Although the fitness constraints imposed by the DL reproductive mode are assumed severe (Ashe & Oldroyd 2002; Hosken & Pitnick 2003; Helms Cahan *et al.* 2004), DL systems are abundant and geographically widespread across the southwestern US (Anderson *et al.* 2006a; Schwander *et al.* 2007a).

In this contribution, we explore the maintenance of DL systems by determining the relative lineage frequencies of 49 DL systems throughout a variety of elevations and habitats representing the entire range of the J1/J2 lineage pair. Owing to the opposing advantages resulting from lineage rarity and ecological factors, we predict that DL systems may be stable despite unequal lineage frequencies and that each system may possess its own unique equilibria driven in part by ecological differences across the range. To test the lasting effect of frequency-dependent selection on worker production, we record early colony growth well beyond the first worker cohort using newly mated queens from four DL systems representing a range of relative lineage frequencies.

Competition for resource space is high within and between *Pogonmyrmex* species (Hölldobler & Wilson 1990). In the field, we determine the association between total colony density and lineage-specific density to discover whether one lineage is a better competitor for resource space. Finally, we employ a previously developed mathematical model that simulates colony growth, survival and reproduction as a function of lineage frequency, assuming random mating and no sperm selection. How would the system respond if one lineage possessed a net ecological advantage that was independent of frequency-dependent effects? We examine how well our model predictions based on a net ecological advantage fit average observed lineage ratios representing the known range of the J1/J2 lineage pair (Fig. 2).

Materials and methods

Study system

The two lineages that comprise each DL system are highly distinct according to multiple classes of molecular markers (Helms Cahan *et al.* 2002, 2004, 2006; Julian *et al.* 2002; Volny & Gordon 2002; Helms Cahan & Keller 2003; Anderson *et al.* 2006a,b; Schwander *et al.* 2006, 2007a,b). Genetic differences indicate that there are two major lineage pairs (Helms Cahan & Keller 2003; Anderson *et al.* 2006a; but see Schwander *et al.* 2007a). The H1/H2 lineage pair occurs primarily in Texas and New Mexico and has the morphology of *Pogonomyrmex rugosus,* while the J1/J2 lineage pair occurs throughout central and southeastern Arizona and resembles *P. barbatus* (Anderson *et al.* 2006a). There are many documented differences between the H1/H2 and J1/J2 lineage pairs (Helms Cahan & Keller 2003; Anderson *et al.* 2006a, 2008, 2009; Schwander *et al.* 2006, 2007a; Helms Cahan *et al.* 2010). For example, the H lineages can be distinguished from one another according to male thorax colour, whereas morphology cannot differentiate the J lineages.

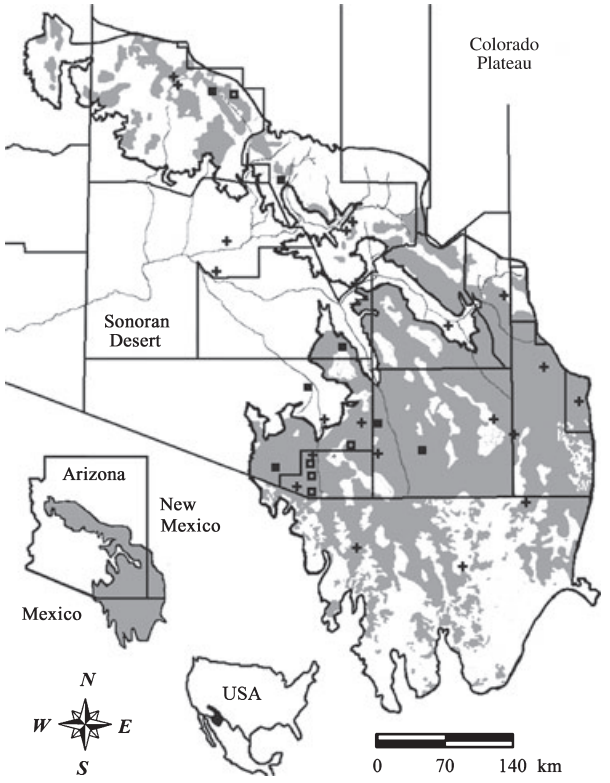

Fig. 2 Map of the Apache Highlands, a highly variable ecoregion that largely defines the present range of the J1/J2 lineage pair (modified from Bailey 1998). Areas in grey are classified as grasslands. The low elevations of the Sonoran desert occur to the Southwest, and the Colorado Plateau is Northeast. Crosses, closed squares, and open squares represent sites where one, two, and three systems were sampled respectively. Refer to Table S1 for details.

On a yearly cycle, *Pogonomyrmex* males emerge synchronously from many colonies, aggregate and release pheromones to attract gynes (male aggregation syndrome; Hölldobler 1976; Hölldobler & Bartz 1985). Aggregations can contain 10^6 reproductives and occur at very high temperatures and humidity 1 or 2 days following a heavy monsoon rain. Reproductive forms tend to aggregate at the same geographical location year after year (Hölldobler 1976; Anderson & Keyel 2006). This site is considered the centre of an effective population (or DL system) and is typically where colony density is greatest (Billick *et al.* 2004). Following mating, the average dispersal distance of J1/J2 queens is <100 m (Suni & Gordon 2009). The colonization success of a newly mated queen is highly dependent on her distance from an established colony (Hölldobler 1981; Wiernasz & Cole 1995). Thus, >99% founding queen mortality is likely due to both temperature extremes and strong competition for nest sites where colony density is high (Wiernasz & Cole 1995; Gordon & Kulig 1996).

Lineage frequencies

We sampled a mean of 56 colonies (range = 36–72) from each of 49 DL systems across central and southeastern Arizona into northern Mexico and western New Mexico (Table S1, Supporting information; Fig. 2). Because the J lineages are morphologically cryptic, we assigned each of the 2747 colonies to lineage J1 or J2 according to restriction fragments of a 650-bp portion of the cytochrome oxidase 1 (*cox1*) MtDNA gene (Anderson *et al.* 2006a). DNA was extracted from one worker head per colony following the protocol outlined in Volny & Gordon (2002) and *cox1* fragments were amplified using the primer pair LCO/HCO according to Anderson *et al.* (2006a). The PCR product was halved and reacted with two different diagnostic restriction enzymes. Lineage J1 *cox1* fragments possess a single restriction site for the enzyme Mfe1, and this site is not present in lineage J2. Lineage J2 possesses a single restriction site for the enzyme Ssp1 and this site is not present in lineage J1.

As an independent confirmation of lineage membership, we sequenced a 650-bp *cox1* fragment for one member of each lineage from all 49 systems for a total of 98 sequences. We compared the unique *cox1* sequence haplotypes from each putative lineage to *cox1* sequences of confirmed J1/J2 systems using a neighbour-joining topology as described by Anderson *et al.* (2006a). We verified the putative lineage designations from the RFLP assays according to the monophyly of *cox1* sequence data with previously confirmed J1 or J2 lineage clades as defined in Helms Cahan & Keller (2003) and Anderson *et al.* (2006a). Although mtDNA sequences from Schwander *et al.* (2007a) were not available for this comparison, the mtDNA-based topology generated from Schwander's broad scale study is entirely congruent with that of both Helms Cahan & Keller (2003), and Anderson *et al.* (2006a,b), particularly concerning the discrimination between lineage J1 and J2. Thus, our phylogenetic analysis provides an independent confirmation of lineage by effectively linking the unique *cox1* sequence haplotypes recovered in this study with results from three separate studies confirming genetic caste determination and J1/J2 lineage membership.

The null hypothesis of equal lineage frequencies (0.50/0.50) was examined with a sign test for each of the 49 systems, and the global lineage frequency was generated by pooling the estimates from all 49 systems.

Environmental heterogeneity

We sampled a range of colony densities and elevations to examine the relationship between relative lineage

frequency and environmental heterogeneity (Table S1, Supporting information). If one lineage holds a competitive or ecological advantage over its partner, the lineage types should be distributed nonrandomly across the landscape with the more competitive of the two lineages relatively overrepresented in areas of high colony density or at different elevations. We sampled systems from a variety of habitats and elevations (351–1580 m) across the known range of the J1/J2 lineage pair (see Anderson *et al.* 2006a; Schwander *et al.* 2007a). Most locales (system breeding centres) were identified using satellite images provided by Google Earth©. To facilitate thermal regulation, mature colonies remove vegetation from around the colony entrance resulting in discs of bare soil approximately 2–6 m in diameter. Competition for resource space between colonies produces a uniform pattern of discs readily identified from satellite photographs. We used a multiple regression model to examine variation in lineage frequency using both elevation and colony density as continuous predictor variables. We used an analysis of covariance to examine the relationship between total colony density and lineage-specific colony density. SAS software was used for all data analysis (SAS Institute Inc 2002–2008)

Colony growth

As demonstrated previously for colony founding and gyne production (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b, 2009; Schwander *et al.* 2006), we predict that colony growth is associated with the relative frequency of each lineage in the system. To this end, we measured differences in colony growth by rearing queens of each lineage under similar laboratory conditions. To distinguish the effects of queen lineage and relative lineage frequency on colony growth, we selected four systems a priori to represent a range of relative lineage frequencies. Newly mated foundress queens were collected following mating aggregations (Table S1, Supporting information; Fig. 5). Queens were sealed in tubes with water-soaked cotton until the first workers emerged, and then test tubes containing surviving colonies were transferred to $12.5 \times 17.5 \times 5.5$ cm plastic boxes or large petri dishes containing an additional water tube. To approximate field conditions, queens were not fed prior to worker emergence. Colonies were maintained at a constant temperature (30 °C) and following the emergence of the first worker cohort were fed ad libitum diet of Kentucky bluegrass seed and crickets once a week. At the end of the experiment, one member of each colony was genotyped to determine lineage membership. Colony growth was recorded as the number of emerged workers at 45, 90 and 180 days. We used *t*-tests to assess differences in worker production between the lineages.

Colony growth model

Using Mathematica 6 (Wolfram Research, Inc 2008), we constructed deterministic and stochastic simulation models that explicitly incorporate mating frequency and other variables affecting colony growth, survival and reproduction (see Anderson *et al.* 2009 for summary of model parameters and values). Specifically, we wanted to determine whether a net ecological advantage to one lineage that affects colony growth, and subsequently the survival of young colonies and the reproduction of established colonies, enables the maintenance of asymmetric lineage frequencies within DL systems. We explicitly modelled differential colony founding and growth because the genetic caste determining mechanism is thought to impose large costs on colony growth and survival, particularly during the production of the first worker cohort. Furthermore, laboratory studies with J1/J2 systems suggest that the growth rate of immature colonies depends not only on the sperm ratios predicted by relative lineage frequency but also on the lineage membership of the queen (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006).

Thus, in our model, colony size was determined exclusively by the genotypes of the queen and her mates, and colony age. We kept track of the proportional representation in the system of distinct classes of colonies defined by age and genotype. We assumed random mating, and the distribution of mating types was modelled deterministically with a binomial distribution. Each generation, the different classes of colonies grew, produced a number of males and gynes depending on colony size and then survived with constant probability. After mating, new queens founded colonies successfully depending on incipient colony size and the number of available nesting spots, i.e. there were density-independent as well as density-dependent components of incipient colony survival. Subsequently, immature colonies grew and joined the pool of established colonies. The dependence of colony growth, survival and reproduction on the proportion of same-lineage mates (see Anderson *et al.* 2009 for details) was the source of the frequency-dependent selection in our model that is characteristic of the DL mutualism. For each generation, the lineage frequency was calculated as the proportional representation of colonies headed by J2 queens. Preliminary trials indicated that 200 generations were a sufficient time to reach a stable population lineage frequency, and all simulations ran 200 generations.

Results

Lineage frequencies

All 49 sampled systems were composed of the J1/J2 lineage pair. Based on 98 *cox1* sequences from across the sampled range, lineage J1 and J2 possessed 18 and 22 unique mtDNA haplotypes, respectively (Table S2, Supporting information). As demonstrated previously (Anderson *et al.* 2006a), *cox1* sequences from each lineage differed dramatically according to a between-group comparison based on nucleotide p-distance (mean ± SE: 0.08 ± 0.013). The *cox1* mtDNA phylogenetic analyses reveal that all putative lineage designations based on the RFLP assays are supported by the inclusion of unique *cox1* haplotypes from this study in monophyletic clades with confirmed lineage membership (Figs S1 and S2, Supporting information).

Lineage frequencies from 25 of 49 (51%) systems differed significantly from equal (Table S1, Supporting information). Lineage J1 was at lower frequency in 39 of 49 systems, and 96% of the distribution in lineage frequencies (47 of 49 systems) occurred between 0.20 and 0.80 (Fig. 3). Pooling colonies across all 49 systems, mean relative lineage frequency differed significantly from equal (sign test, $P < 0.0001$, $N = 2747$ colonies, mean frequency of lineage J1 = 0.397, 95% CI = 0.378–0.416).

Environmental heterogeneity

The regression model with relative lineage frequency as the dependent variable and elevation and total colony density (combined density of lineage J1 and J2) as predictor variables was significant ($r^2 = 0.41$, $F_{2,46} = 15.7$, $P < 0.0001$). Beta coefficients indicate a negative relationship between relative lineage frequency and total colony density ($\beta = -0.39$) and a positive relationship between relative lineage frequency and elevation ($\beta = 0.53$). Thus, lineage J1 becomes relatively less frequent as total colony density increases and relatively more frequent as elevation increases (Fig. 4). As total colony density increases, the density of lineage J2 increases at a significantly greater rate than the density of lineage J1 (Fig. 5, $F = 66.6$, $P < 0.0001$). Consistent with these findings, the density of lineage J2 increases with its relative frequency ($r^2 = 0.40$, $F_{1,48} = 30.7$, $P < 0.0001$), but there was no relationship between lineage J1 density and relative lineage frequency ($r^2 = 0.07$, $F_{1,48} = 3.6$, $P < 0.06$).

Elevation and total colony density were unrelated ($r^2 = 0.03$, $F_{1,47} = 0.21$, $P < 0.65$). A lineage-specific analysis also reveals that elevation is unassociated with the density of lineage J2 colonies (Fig. 5, $r^2 = 0.01$, $F_{1,47} = 0.54$, $P < 0.47$). However, the relationship between ele-

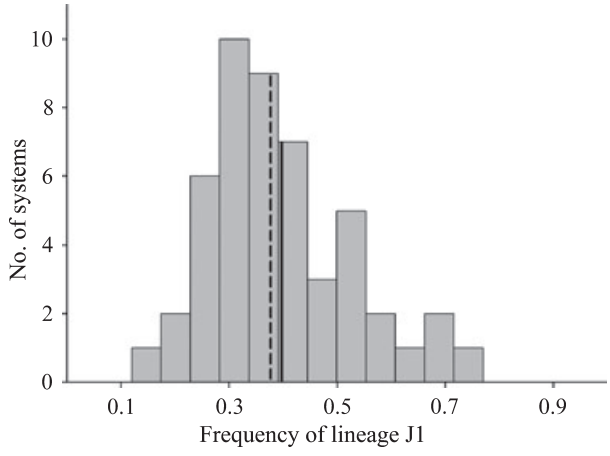

Fig. 3 A histogram of J1 lineage frequencies based on 49 systems sampled throughout the known range of the J1/J2 lineage pair. Mean frequency is 0.397 (solid line) and median frequency is 0.375 (dashed line). Lineage frequencies are relative; i.e. the frequency of lineage J2 is equal to one minus the frequency of lineage J1.

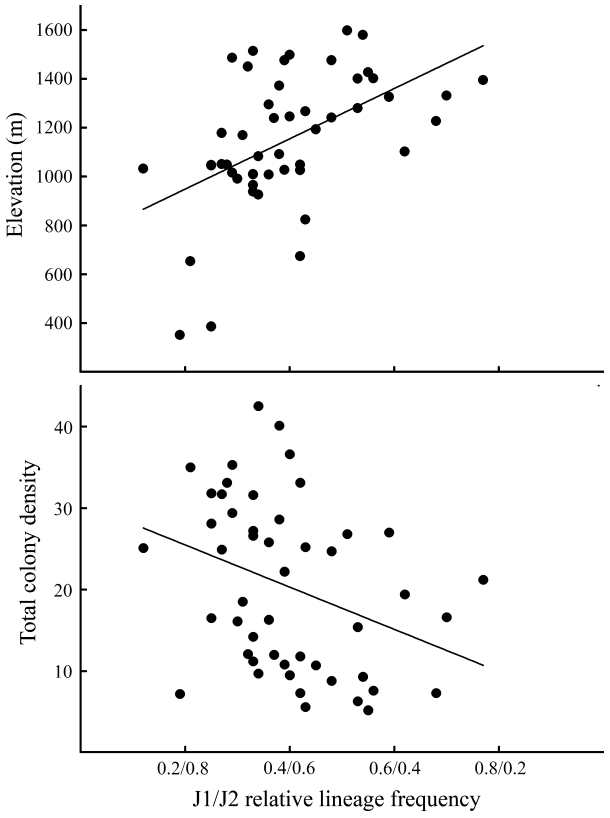

Fig. 4 The relationship of relative lineage frequency (*x*-axis) with total colony density (lower panel) and elevation (upper panel). Both regressions differ significantly from zero slope; Colony density: $r^2 = 0.13$, $F = 6.7$, $P < 0.01$, Elevation: $r^2 = 0.26$, $F = 16.1$, $P < 0.0002$.

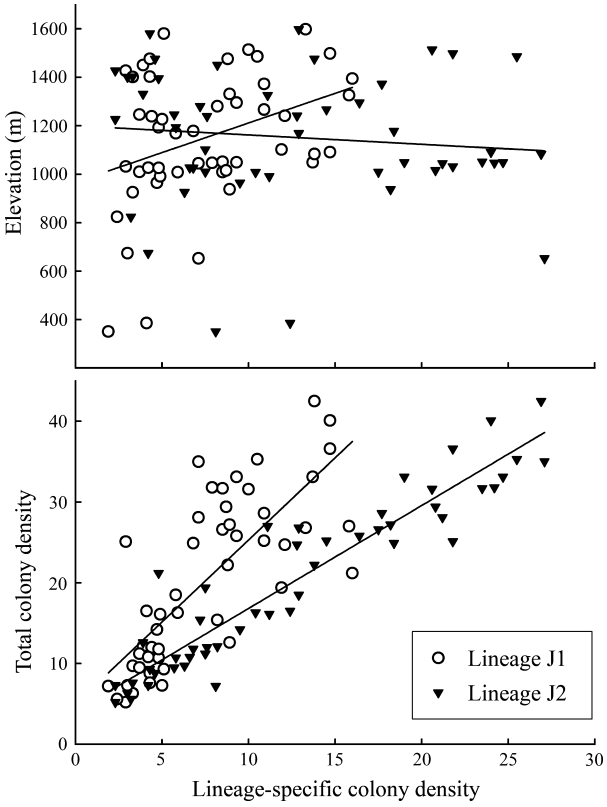

Fig. 5 The relationship of lineage-specific colony density (*x*-axis) with total colony density (lower panel) and elevation (upper panel). The density of lineage J1 is associated with elevation ($r^2 = 0.12$, $F_{1,47} = 6.5$, $P < 0.01$), a relationship solely attributable to the five systems under 900 m (see Results). Both regression lines in the lower panel differ significantly from a slope of zero. As total colony density increases, the density of lineage J2 increases at a greater rate than the density of lineage J1 ($F = 66.6$, $P < 0.0001$).

vation and lineage J1 colony density differs significantly from a slope of zero, but explains very little of the variance in the data set ($r^2 = 0.12$, $F_{1,47} = 6.5$, $P < 0.014$). This trend was driven by the five systems under 900 m where both the relative frequency and density of lineage J1 are low (Figs 4 and 5). A subsequent analysis excluding the five systems below 900 m ($n = 44$) reveals no relationship between elevation and lineage J1 colony density ($r^2 = 0.03$, $F_{1,42} = 1.4$, $P < 0.25$).

Colony growth

We measured differences in colony growth as a result of the effect of queen lineage (genotype) and relative lineage frequency (Fig. 6). Consistent with previous experiments (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006), J1 queens from systems in which the J1 lineage was rare all showed significantly greater worker production than did lineage J2 at

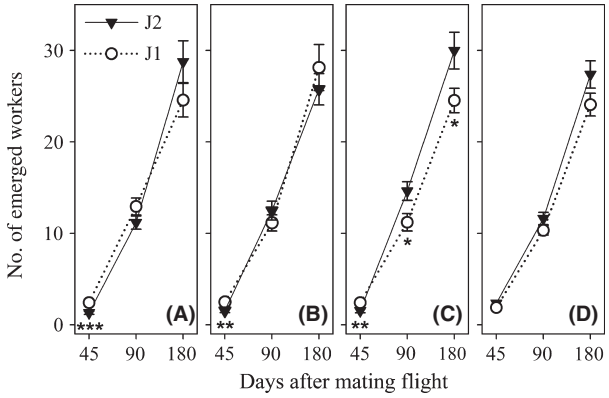

Fig. 6 Mean colony growth in four J1/J2 systems that differ in relative lineage frequency. Whiskers are standard errors. Relative lineage frequencies (J1/J2) are: panel A; (0.19/0.81), panel B; (0.28/0.72), panel C; (0.39/0.61), and panel D; (0.53/0.47). Queen sample sizes for each system and lineage (J1, J2) were A; (33, 58), B; (31, 44), C; (40, 46) and D; (45, 41). Asterisks designate significant differences in worker production between queens of each lineage based on *t*-tests ($P < 0.0001$***, $P < 0.005$**, and $P < 0.05$*). The first worker cohort (45 days) was produced prior to food supplementation.

45 days (Fig. 6, J1/J2 mean ± SE: Panel A: 2.4 ± 0.2/1.3 ± 0.2, Panel B: 2.5 ± 0.3/1.5 ± 0.2, Panel C: 2.4 ± 0.2/1.5 ± 0.2). This trend did not continue after colonies with emerged workers were provided nutritional resources. At 180 days, lineage J2 had produced more workers than lineage J1 in three of the four systems regardless of lineage frequency. However, this difference was significant in only one of the three systems (Fig. 6, J1/J2 mean ± SE, Panel C; 24.5 ± 1.3/30.0 ± 2.0). In the system with relatively equal lineage frequencies, J2 grew slightly faster than lineage J1 but there was no significant difference in colony growth at 45, 90 or 180 days (mean ± SE number of workers in linage J1/J2 at 45 days: 1.9 ± 0.2/2.3 ± 0.2, 90 days: 10.3 ± 0.5/11.6 ± 0.7, 180 days: 24.1 ± 1.2/27.4 ± 1.5).

Colony growth model

We found that an ecological advantage of colonies headed by queens of one lineage produced a stable lineage frequency that was asymmetric (Fig. 7). The ecological advantage influenced colony size, which affected the survival during colony founding as well as reproduction of established colonies. These two effects of the ecological advantage (*a*) had additive effects on lineage frequency asymmetry, but consistent with previous empirical results (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006), the effect on survival during colony founding was relatively more important. In both cases, the ecological advantage partially offsets the costs to the common lineage imposed

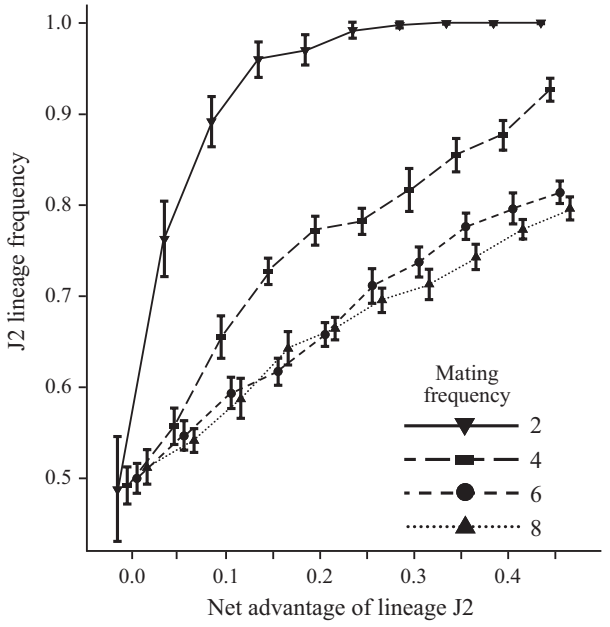

Fig. 7 A stochastic model depicting the relationship between J2 lineage frequency and a net ecological advantage for lineage J2 which affects both initial survival and subsequent reproduction. The four plots represent queens mating randomly with 2, 4, 6, or 8 males (top down). The error bars are 95% confidence intervals for the mean frequency across 20 runs. Each simulation ran for 200 generations, and survival of colonies after establishment was 0.9. A deterministic version of the model produced similar results. See Anderson *et al.* (2009) for a detailed description of model parameters.

by the negative frequency-dependent selection associated with colony founding success. Parameters determining mating frequency (M) and the survival of established colonies (s) also affected the stable lineage frequencies. Systems with lower mating frequency had more extremely biased lineage frequencies (Fig. 7). Systems in which established colonies suffered higher mortality (i.e. lower s) also had more extremely biased lineage frequencies (data not shown). The magnitude of lineage asymmetry observed in our system survey was relatively easy to achieve in the simulation model. For example, in our simulation model, the mean frequency of J1 actually observed across all systems ($\sim$0.4) could be achieved with approximately a 12% net ecological advantage to colonies with J2 queens (Fig. 7).

Discussion

Like obligate mutualisms, DL systems require the continued interaction and co-evolution of two reproductively isolated lineages. It was previously demonstrated that queens from a rare lineage will mate primarily with alternate-lineage males, acquire mostly worker-destined sperm and produce more workers in the first cohort (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006). The strength of this frequency-dependent selection pressure depends on the relative frequency of each lineage in the system, and when all other factors are held constant between the lineages, the increase in rare-lineage worker production should act to increase the frequency of the rare lineage until lineage frequencies stabilize near 0.5/0.5 (Yamauchi & Yamamura 2006). By deduction, lineage frequencies from a large sample of DL systems should result in a pooled distribution wherein the mean relative lineage frequencies are indistinguishable from 0.5/0.5. However, our empirical results demonstrate that the global J1/J2 relative lineage frequency, 0.4/0.6, differs significantly from equal (Fig. 3), a result inconsistent with theoretical expectations of converging lineage frequencies. Colonies headed by J2 queens are more frequent than colonies headed by J1 queens over most of the sampled geographical range, suggesting that J2 possesses a net ecological advantage that is largely independent of balancing selection or environmental heterogeneity. Thus, we propose that asymmetrical lineage frequencies may represent a stable equilibrium between a net ecological advantage intrinsic to lineage J2 and the consequent advantage of rarity bestowed upon lineage J1.

Colony growth

Results from the growth rate experiments suggest that following the extreme selection associated with producing the first worker cohort, the common lineage (usually J2) can compensate for less than optimal sperm ratios via intrinsic growth differences (Helms Cahan *et al.* 2010), which may also be supplemented by the capacity of lineage J2 to recognize and nurture appropriate genotypes (Clark *et al.* 2006). Our experiments examining colony growth under laboratory conditions demonstrate that the deficiencies suffered by the first brood cohort of the common lineage in this and other experiments (Anderson *et al.* 2006b; Schwander *et al.* 2006) did not translate into a long-term growth difference between the lineages. Following the production of the first brood cohort, colonies headed by J2 queens outgrew lineage J1 in three of the four experiments regardless of the relative lineage frequencies of the particular system (Fig. 6). Significant differences between the J lineages in the conversion of eggs to pupae, and fecundity (Helms Cahan *et al.* 2010) provide mechanisms for the growth rates we observed. Lineage J1 more efficiently converts eggs to viable pupae, a frequency-dependent mechanism resulting from lineage J1 rarity. This occurs because the majority of rare-lineage (J1) eggs are randomly fertilized with common-lineage

(worker-destined) sperm. This advantage applies particularly to the first worker cohort, which is raised entirely on the energetic reserves of the founding queen. Conversely, lineage J2 has a fecundity advantage that is independent of lineage frequency. But this advantage only applies following the emergence of the first worker cohort when foraging provides nutritional input (seeds and insects) to support the greater fecundity of J2 queens. Three separate studies have now demonstrated higher fecundity in lineage J2 queens (Schwander *et al.* 2006, 2007a; Helms Cahan *et al.* 2010), results consistent with the hypothesis of an intrinsic J2 growth advantage.

Mating frequency is another intrinsic factor that may explain the higher frequency of lineage J2 across the range and in areas of high colony density. Lineage J2 queens tend to mate with more males on average than lineage J1 queens ($J2 \approx 12$, $J1 \approx 8$; Brendon Mott, unpublished data). Thus, while lineage J2 typically acquires proportionately less worker-destined sperm than lineage J1, an increase in J2 mating frequency when the lineages are in typical proportions (0.6/0.4) would provide J2 colonies with similar worker patriline diversity relative to lineage J1, a trait demonstrated to increase colony growth and foraging time in a well-studied congener (Cole & Wiernasz 1999; Cole *et al.* 2010). Colonies with greater genetic diversity foraged for longer time periods in the morning than did colonies with less diversity, suggesting that a genetically diverse workforce is ecologically superior (Cole *et al.* 2010). Competitive advantages attributed to a diverse workforce are well established in the honeybee (Mattila & Seeley 2007; Eckholm *et al.* 2010) and have been modelled as 'social heterosis' by Nonacs & Kapheim (2007), providing a mechanism by which DL systems may maintain genetic diversity. In addition to social heterosis, lineage advantages associated with mitochondrial DNA or cytonuclear combinations in F_1 workers may affect colony growth, adaptation to thermal regimes and future reproduction (Linksvayer *et al.* 2006). Faster colony growth translates into an ability to dominate resources and territory (Oster & Wilson 1978) and may account in part for the relative overabundance of lineage J2 from areas of high colony density where competition between neighbours is maximal (Fig. 5).

Colony density

Positive density dependence acts to keep the J1/J2 system tied to a particular patch where soils provide desiccation resistance, resources are abundant and queens can easily find and mate with both male lineage types. The reproductive strategy of a DL system is consistent with these requirements; reproductive forms aggregate at areas of high colony density, and newly mated queens disperse an average of <100 m (Suni & Gordon 2009) such that the vast majority of new colonies are initiated in the preferred habitat. However, at densities above 20 colonies per hectare, J1/J2 systems begin to show uniform dispersion patterns indicative of extreme competition. Uniform colony distributions are common in ants (Levings & Traniello 1981) and in *Pogonomyrmex* are thought to arise by 'self-thinning', a process associated with the nonrandom colony mortality of neighbours of different age classes owing to either resource competition (Davidson 1985) or direct interference competition (Wiernasz & Cole 1995).

Our results suggest that lineage J1 seems to coexist spatially with lineage J2 at lower total colony densities that typically represent the fringes of a breeding centre (Fig. 5). However, near breeding centres where total colony density is high, lineage J2 dominates resource space, possibly influencing the way in which relative lineage frequencies are maintained by balancing selection. The frequency-dependent advantage of J1 rarity may not apply to newly mated queens that initiate colonies in areas of high J2 density. Lineage J1 colonies may simply be outcompeted very early in their development due to the J2 fecundity advantage. Thus, most of the competition in areas of high colony density likely occurs within lineage J2 among larger adolescent colonies and reproductively capable colonies. Under this scenario, the net cost to lineage J1 would be low, but continual competition between large neighbouring colonies of lineage J2 may reduce the number of J2 colonies that attain reproductive maturity and decrease the total reproductive output of mature J2 colonies.

Elevation

The lineages J1 and J2 are highly differentiated genetically (Helms Cahan & Keller 2003; Anderson *et al.* 2006a; Schwander *et al.* 2006) such that the performance of each lineage may vary according to elevation-associated climatic factors. The primary range of the J1/J2 lineage pair occurs just above the convoluted northeastern border of the Sonoran desert (above 900 m; Shreve 1951), where increasing precipitation supports less arid types of vegetation. Most J1/J2 systems occupy semi-desert grasslands or riparian areas of the Apache Highlands Ecoregion (Bailey 1995, 1998), occurring sporadically below the Mogollon Rim, and becoming more common throughout southern habitats associated with sky islands (Fig. 2). Grasslands abound at these higher elevations, supporting large meta-systems, where J1/J2 colonies are overdispersed across the landscape for many miles and likely have thousands of different centres of breeding activity (mating aggregation areas). In contrast, the DL systems below 900 m

are small and isolated with only a few centres of breeding activity.

Both relative lineage frequency and the density of lineage J1 were associated with elevation, suggesting that lineage J1 is better adapted to climatic conditions above 900 m (Figs 4 and 5). The climate across the low-elevation Sonoran desert region became much warmer and drier following the last glacial maximum some 18 kya, replacing grasslands with more arid-tolerant shrubs (Thompson & Anderson 2000). The five J1/J2 systems occurring below 900 m (Fig. 5) were intimately tied to areas with irrigated agricultural soils or areas where high water tables were associated with natural or artificial drainage systems. The J1/J2 lineage pair is associated with soil types that limit desiccation (Johnson 2000), such that the ability to disperse to a physiological range amenable to both lineages may be limited by the availability and proximity of adequate soils and access to the water table. The effects of drift become more prominent in smaller populations, and combined with low dispersal distances and elevation-associated selection pressure on lineage J1, likely contributed to the localized extinction of many J1/J2 systems throughout the Sonoran desert.

Are skewed lineage frequencies stable?

Because efficient worker production is required for colony maintenance and growth to a mature size, only those queens possessing sufficient worker-destined sperm will contribute genes to the next generation (by producing either haploid males or diploid queens). Our simulation modelled colony growth and reproduction (see Anderson *et al.* 2009). The advantage to colonies headed by J2 queens affected colony size, which in turn affected the survival rate of incipient colonies and the reproductive output of established colonies. As predicted by mathematical models and demonstrated empirically, common-lineage colonies that survive to reproductive maturity produce more gynes relative to the rare lineage (Yamauchi & Yamamura 2006; Anderson *et al.* 2009; Helms Cahan *et al.* 2010). Both of these effects influenced stable lineage frequencies by partially offsetting the advantage of the rare lineage during early colony founding. The cost to the common lineage on early colony survival is thought to be particularly important (Helms Cahan *et al.* 2004; Anderson *et al.* 2006b; Schwander *et al.* 2006), and our simulation model confirmed these results. The effect of the ecological advantage on stable lineage frequency depended on both mating frequency and the survival rate of established colonies, such that systems with a lower mating frequency and with lower survival of established colonies had more unequal lineage frequencies (Fig. 7). With low mating frequency, many queens (particularly common-lineage queens) mate with only same-lineage males and fail to found colonies. As the mating frequency increases, this load is spread more evenly across all colonies (Fig. 7). Thus, both mating frequency and the survival rate of established colonies affect the strength of selection on the system and the stable lineage frequency that is maintained.

With realistic mating frequencies (>8) and survival rates, our model suggests that a 12% net ecological advantage to J2 queens relative to J1 queens could produce the average lineage frequency asymmetries we observed in our system survey (Fig. 7). Because selection is strong when colonies are small (Oster & Wilson 1978), an early fecundity advantage for lineage J2 may contribute markedly to the unequal lineage frequencies we observed (Fig. 3). As detailed above, the net ecological advantage may result from some combination of queen traits such as fecundity, thermal tolerance, or mating frequency, or with worker traits that may depend on cytoplasmic factors contributed by the queen, like worker development rate or metabolic thresholds for temperature tolerance. Regardless of the contributing mechanisms, our simulations demonstrate that a net ecological advantage to colonies headed by J2 queens could produce the lineage frequencies we observed, and these unequal lineage frequencies could be stably maintained. The strength of ecological advantage may also differ geographically such that each system may possess its own unique equilibria. Thus, the lineage frequencies documented across 49 systems may all represent relatively stable lineage frequencies, and the DL system may not be as constrained to symmetric lineage frequencies as predicted previously (Yamauchi & Yamamura 2006).

The relative lack of systems containing lineage frequencies below 0.2 (Fig. 3) suggests a limit on the range of relative lineage frequencies that can be maintained in nature. If one lineage holds a large net advantage such that lineage frequencies become extremely unequal, the rare-lineage advantage also diminishes owing to drastic reductions in gyne production. This finding is consistent with empirical results wherein gyne production by rare-lineage queens decreased sharply with increased lineage rarity (Anderson *et al.* 2009; Helms Cahan *et al.* 2010). Thus, extremely unequal lineage frequencies may result in the localized extinction of the system owing to the unavailability of rare-lineage queens.

Differences between J1/J2 and H1/H2

Many differences are apparent when comparing the H1/H2 lineage pair with *P. rugosus*-like morphology to the J1/J2 lineage pair with *P. barbatus*-like morphology. These lineage pairs differ broadly based on genetic

analyses and distribution patterns (Anderson *et al.* 2006a; Schwander *et al.* 2007a). In the H1/H2-dependent lineages, sign tests indicate that 8 of 20 systems differ significantly from equal lineage frequencies (data from Schwander *et al.* 2007b). The weight of the pooled distribution is centred on the mean, and mean relative lineage frequency is not distinguishable from 0.5/0.5 (sign test; $P = 0.26$, $N = 903$ colonies, mean frequency of lineage H1 = 0.519, 95% CI = 0.487–0.552). In contrast to our findings for the J1/J2 lineage pair, the mean relative lineage frequency of the H1/H2 lineage pair is consistent with the convergence of lineage frequencies towards 0.5/0.5. This result suggests that neither of the lineages possess a net advantage and that frequency-dependent selection is a primary stabilizing force of the H1/H2 system. Thus, while one laboratory experiment found that newly mated queens of lineage H2 had greater fecundity than lineage H1 queens (Schwander *et al.* 2006), this growth difference is not reflected in the relative lineage frequencies of field systems. Also, H1/H2 systems were sampled from a wide variety of elevations and habitats, but their relative lineage frequencies were unassociated with elevation. These findings indicate that the J1/J2 lineage pair has co-evolved differently than the H1/H2 lineage pair for traits that are fundamental to DL system maintenance (Anderson *et al.* 2009).

DL system evolution

As seen in obligate dispersive mutualisms (Holland & DeAngelis 2001), DL system dynamics are strongly influenced by the relative number of individuals of each species or lineage, emphasizing the importance of frequency-dependent selection in models of co-evolution (Levin & Udovic 1977). DL systems involve a dynamic relationship between frequency-dependent and frequency-independent forces, potentially generating many different localized and independently evolving system equilibria. The range of the J1/J2 DL pair is broad, disjointed and occupies a variety of elevations and communities, features that make the system particularly suited for investigating co-evolution in response to a selection mosaic (see Thompson 1999; Gomulkiewicz *et al.* 2000). Because the lineages are genetically distinct, intrinsic differences or environmental heterogeneity can result in the local maladaptation of one or both of the interacting lineages (Thompson 1999). Colony fitness in a DL system relies on continued interaction between two reproductively isolated gene pools, a circumstance that may constrain the adaptive evolution of both lineages (Anderson *et al.* 2008). The lineages mate at centralized aggregations and are limited in their dispersal ability, traits that may accelerate the effects of inbreeding and genetic drift (see Cole & Wiernasz 1997). To counter these effects, an unusually high mating frequency results in a colony of genetically diverse individuals whose interaction generates group fitness benefits or 'social heterosis' (Nonacs & Kapheim 2007), a process predicted to maintain genetic diversity for the expression of many adaptive phenotypes, both behavioural and reproductive.

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References

Anderson KE, Keyel AC (2006) Mating flight, metrosis and semi-claustrality in the seed-harvester ant *Pogonomyrmex salinus* (Hymenoptera: Formicidae). *Insectes Sociaux*, **53**, 92–96.

Anderson KE, Gadau J, Mott BM *et al.* (2006a) Distribution and evolution of genetic caste determination in *Pogonomyrmex* seed-harvester ants. *Ecology*, **87**, 2171–2184.

Anderson KE, Holldobler B, Fewell J, Mott BM, Gadau J (2006b) Population-wide lineage frequencies predict genetic load in the seed-harvester ant *Pogonomyrmex*. *Proceedings of the National Academy of Science, USA*, **103**, 13433–13438.

Anderson KE, Linksvayer TA, Smith CR (2008) Causes and consequences of genetic caste determination in ants. *Myrmecological News*, **11**, 119–132.

Anderson KE, Smith CR, Linksvayer TA *et al.* (2009) Modeling the maintenance of a dependent lineage system: the influence of positive frequency dependent selection on sex ratio. *Evolution*, **63**, 2142–2152.

Ashe A, Oldroyd B (2002) Genetic determination of caste in harvester ants. *Trends in Ecology and Evolution*, **17**, 448–449.

Bailey RG (1995) *Descriptions of the Ecoregions of the United States*, 2nd edn. U.S.D.A. Forest Service Miscellaneous Publication 1391, Washington, District of Columbia.

Bailey RG (1998) *Ecoregions Map of North America: Explanatory Note*. U.S.D.A. Forest Service Miscellaneous Publication 1548, Washington, District of Columbia.

Billick I, Cole BJ, Wiernasz DC (2004) Scale of recruitment limitation in the western harvester ant (Hymenoptera: Formicidae). *Annuals of the Entomological Society of America*, **97**, 738–742.

Clark RM, Anderson KE, Gadau J, Fewell JH (2006) Behavioral regulation of genetic caste determination in a *Pogonomyrmex* population with dependent lineages. *Ecology*, **87**, 2201–2206.

Cole BJ, Wiernasz DC (1997) Inbreeding in a lek-mating ant species, *Pogonomyrmex occidentalis*. *Behavioral Ecology and Sociobiology*, **40**, 79–86.

Cole BJ, Wiernasz DC (1999) The selective advantage of low relatedness. *Science*, **285**, 891–893.

Cole BJ, Smith AA, Huber ZJ, Wiernasz DC (2010) The structure of foraging activity in colonies of the harvester ant, *Pogonomyrmex occidentalis*. *Behavioral Ecology*, **21**, 337–342.

Curry MM, Wheeler DE, Yang K, Anderson KE (2009) The potential for gene flow in a dependent lineage system of a Harvester ant: Fair meiosis in the F1 generation. *Journal of Heredity*, **101**, 378–384.

Davidson DW (1985) An experimental study of diffuse competition in harvester ants. *American Naturalist*, **125**, 500–506.

Eckholm BJ, Anderson KE, Weiss M, DeGrandi-Hoffman G (2010) Intracolonial genetic diversity in honeybee (*Apis mellifera*) colonies increases pollen foraging efficiency. *Behavioral Ecology and Sociobiology*, doi:10.1007/s00265-010-1108-8.

Gomulkiewicz R, Thompson JN, Holt RD, Nuismer SL, Hochberg ME (2000) Hot spots, cold spots, and the geographic mosaic theory of coevolution. *American Naturalist*, **156**, 156–174.

Gomulkiewicz R, Nuismer SL, Thompson JN (2003) Coevolution in variable mutualisms. *American Naturalist*, **162**, S80–S93.

Gordon DM, Kulig AW (1996) Founding, foraging, and fighting: colony size and the spatial distribution of harvester ant nests. *Ecology*, **77**, 2293–2409.

Helms Cahan S, Julian GE (2010) Shift in frequency-dependent selection across the life-cycle in obligately interbreeding harvester ant lineages. *Evolutionary Ecology*, **24**, 359–374.

Helms Cahan S, Keller L (2003) Complex hybrid origin of genetic caste determination in harvester ants. *Nature*, **424**, 306–309.

Helms Cahan S, Parker JD, Rissing SW *et al.* (2002) Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. *Proceedings of the Royal Society of London, Series B*, **269**, 1871–1877.

Helms Cahan S, Julian GE, Rissing SW *et al.* (2004) Loss of phenotypic plasticity generates genotype-caste association in harvester ants. *Current Biology*, **14**, 2277–2282.

Helms Cahan S, Julian GE, Schwander T, Keller L (2006) Reproductive isolation between *Pogonomyrmex rugosus* and two lineages with genetic caste determination. *Ecology*, **87**, 2160–2170.

Helms Cahan S, Daly AB, Schwander T, Woods HA (2010) Genetic caste determination does not impose growth rate costs in *Pogonomyrmex* harvester ants. *Functional Ecology*, **24**, 301–309.

Holland JN, DeAngelis DL (2001) Population dynamics and the ecological stability of obligate pollination mutualisms. *Oecologia*, **126**, 575–586.

Hölldobler B (1976) The behavioral ecology of mating in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). *Behavioral Ecology and Sociobiology*, **1**, 405–423.

Hölldobler B (1981) Foraging and spatiotemporal territories in the honey ant *Myrmeeocystus mimicus* Wheeler (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology*, **9**, 310–314.

Hölldobler B, Bartz SH (1985) The sociobiology of reproduction in ants. In: *Experimental Behavioral Ecology and Sociobiology* (eds Hölldobler B, Lindauer M), pp. 237–257, Sinauer Associates, Sunderland.

Hölldobler B, Wilson EO (1990) *The Ants*. Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Hölldobler B, Wilson EO (2008) *The Superorganism*. Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Hosken DJ, Pitnick S (2003) Do queens select sperm? *Trends in Ecology and Evolution*, **18**, 107.

Hughes WOH, Boomsma JJ (2008) Genetic royal cheats in leaf-cutting ant societies. *Proceedings of the National Academy of Science, USA*, **103**, 5150–5153.

Johnson RA (2000) Habitat segregation based on soil texture and body size in the seed- harvester ants *Pogonomyrmex rugosus* and *P. barbatus*. *Ecological Entomology*, **25**, 403–412.

Julian GE, Fewell JH, Gadau J, Johnson RA, Larrabee D (2002) Genetic determination of the queen caste in an ant hybrid zone. *Proceedings of the National Academy of Science, USA*, **99**, 8157–8160.

Levin SA, Udovic JD (1977) A mathematical model of coevolving populations. *American Naturalist*, **111**, 657–675.

Levings SC, Traniello JFA (1981) Territoriality, nest dispersion, and community structure in ants. *Psyche*, **88**, 265–319.

Linksvayer TA, Wade MJ, Gordon DM (2006) Genetic caste determination in harvester ants: possible origin and maintenance by cyto-nuclear epistasis. *Ecology*, **87**, 2185–2193.

Mattila HR, Seeley TD (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. *Science*, **317**, 362–364.

Nonacs P, Kapheim KM (2007) Social heterosis and the maintenance of genetic diversity. *Journal of Evolutionary Biology*, **20**, 2253–2265.

Nuismer SL, Gomulkiewicz R, Morgan MT (2003) Coevolution in temporally variable environments. *American Naturalist*, **162**, 195–204.

Oster GF, Wilson EO (1978) *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, New Jersey, 352 pp.

Parker JD (2004) A major evolutionary transition to more than two sexes? *Trends in Ecology and Evolution*, **2**, 83–86.

SAS Institute Inc (2002–2008) *Version 9.2*. SAS Institute Inc.,Cary, North Carolina.

Schwander T, Helms Cahan S, Keller L (2006) Genetic caste determination in *Pogonomyrmex* harvester ants imposes costs during colony founding. *Journal of Evolutionary Biology*, **19**, 402–409.

Schwander T, Helms Cahan S, Keller L (2007a) Characterization and distribution of *Pogonomyrmex* harvester ant lineages with genetic caste determination. *Molecular Ecology*, **16**, 367–387.

Schwander T, Keller L, Helms Cahan S (2007b) Two alternate mechanisms contribute to the persistence of interdependent lineages in *Pogonomyrmex* harvester ants. *Molecular Ecology*, **16**, 3533–3543.

Schwander T, Lo N, Beekman M, Oldroyd P, Keller L (2010) Nature versus nurture in social insect caste differentiation. *Trends in Ecology and Evolution*, **25**, 275–282.

Shreve F (1951) *Vegetation of the Sonoran Desert*. Carnegie Institution of Washington, Pub. 591, Washington, 192 pp.

Smith CR, Anderson KE, Tillberg CV, Gadau J, Suarez AV (2008) Caste determination in a polymorphic social insect: nutritional, social and genetic factors. *American Naturalist*, **172**, 497–507.

Suni SS, Gordon DM (2009) Fine-scale genetic structure and dispersal distance in the harvester ant *Pogonomyrmex barbatus*. *Heredity*, **104**, 168–173.

Thompson JN (1999) The evolution of species interactions. *Science*, **284**, 2116–2118.

Thompson RS, Anderson KH (2000) Biomes of western North America at 18,000, 6000 and 0 14C yr bp reconstructed from pollen and packrat midden data. *Journal of Biogeography*, **27**, 555–584.

Travis J (1996) The significance of geographic variation in species interactions. *American Naturalist*, **148**, S1–S8.

Volny VP, Gordon DM (2002) Genetic basis for queen-worker dimorphism in a social insect. *Proceedings of the National Academy of Science, USA*, **99**, 6108–6111.

Wheeler DE (1986) Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *American Naturalist*, **128**, 13–34.

Wiernasz DC, Cole BJ (1995) Spatial distribution of *Pogonomyrmex occidentalis*: recruitment, mortality and overdispersion. *Journal of Animal Ecology*, **64**, 519–527.

Wolfram Research, Inc (2008) *Mathematica, Version 6*. Champaign, Illinois.

Yamauchi A, Yamamura N (2006) Persistence of symmetrical social hybridogenesis in haplodiploid Hymenoptera. *Journal of Theoretical Biology*, **240**, 24–31.

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Data accessibility

DNA sequences: GenBank accessions JF267717–JF267772, AY542355, AY542356, AY542358, AY542359, AY542361, AY542362, AY542364, AY542368, AY542370. See Table S2 (Supporting information) for related information.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 A mtDNA (415-bp partial *cox1*) topology estimated by neighbour joining with 1000 bootstraps and *Pogonomyrmex californicus* (*P. calif*) as the outgroup.

Fig. S2 An analysis of lineage J2 using the same reference sequences and parameters used to generate Figure S1.

Table S1 Location, sample size, colony density and lineage frequencies of 49 J1/J2-dependent lineage systems.

Table S2 Genbank accession numbers and sequence ID's of *cox1* mitochondrial gene fragments used to construct neighbour-joining topologies (Figs S1 and S2).

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