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MODELING THE MAINTENANCE OF A DEPENDENT LINEAGE SYSTEM: THE INFLUENCE OF POSITIVE FREQUENCY-DEPENDENT SELECTION ON SEX RATIO

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In insect societies, worker versus queen development (reproductive caste) is typically governed by environmental factors, but some *Pogonomyrmex* seed-harvester ants exhibit strict genetic caste determination, resulting in an obligate mutualism between two reproductively isolated lineages. Queens mate randomly with multiple males from each lineage and intralineage crosses produce new queens, whereas interlineage crosses produce workers. Early colony survival is negatively frequency dependent; when lineage frequencies are unequal, queens from the rarer lineage benefit because they acquire more interlineage sperm, and produce more workers. Here we examine theoretically and empirically the effect of relative lineage frequency on sex ratio. We predict that the ratio of inter- to intralineage sperm acquired by queens of each lineage will affect the sex ratio produced at colony maturity. Consistent with model predictions, we found that gyne production in mature colonies was positively frequency dependent, increasing significantly with increasing lineage frequency across 15 populations. Unequal lineage frequencies are common and likely maintained by a complex interplay between an ecological advantage specific to one lineage, and opposing frequency-dependent selection pressures experienced throughout the colonies life-cycle; rare lineage colonies benefit during early colony growth, and common lineage colonies benefit at reproductive maturity.

KEY WORDS: Dependent lineage, frequency-dependent selection, inclusive fitness, obligate mutualism, polyphenism, sex allocation, symmetrical social hybridogenesis.

In most eusocial ants, wasps, and bees, queen-worker caste determination is a plastic developmental process governed by the type or amount of nutrition that workers provide to developing diploid individuals (Wheeler 1986; Smith et al. 2008a). In certain insect societies however, worker control of caste determination is reduced because caste is determined mainly by larval genotype and less influenced by rearing conditions (Anderson et al. 2008; Smith et al. 2008b). An extreme example is found in many populations of the seed-harvester ants *Pogonomyrmex barbatus* and *P. rugosus* (reviewed in Anderson et al. 2008). Here, female

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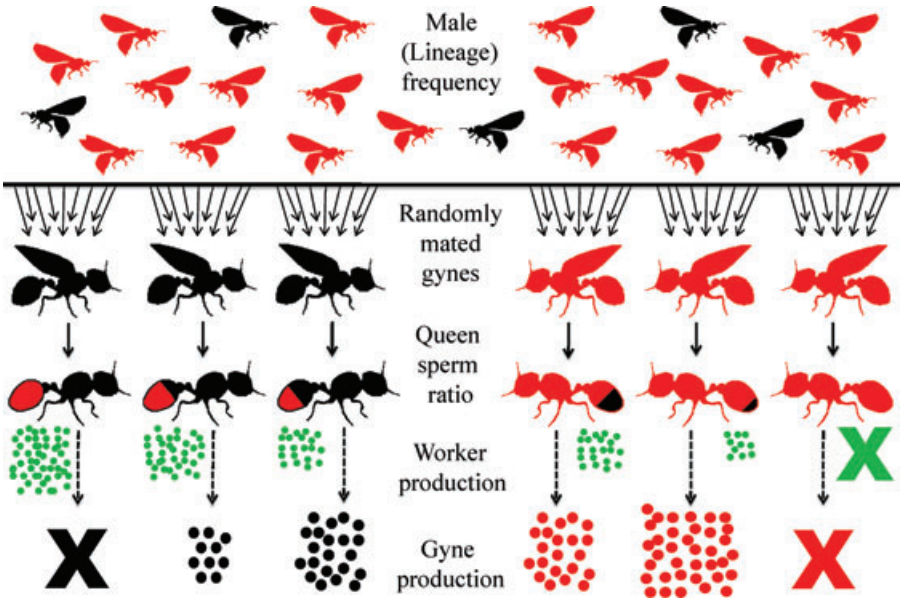

Figure 1. **A diagram depicting fitness asymmetry in a dependent lineage population as a result of unbalanced lineage frequencies. Black and red represent the rare and common lineage, respectively. Small green dots are F_1 worker production and large dots are gyne production. At the top of the diagram, queens mate randomly with multiple males from each lineage. This results in predictable fitness classes (columns) determined by sperm ratios stored in the queen. Queen sperm ratios are depicted as the proportion of the gaster (oval) filled with alternate-lineage (worker-destined) sperm. In colonies that attain reproductive maturity, male production is essentially constant among the fitness classes, so fitness differences are primarily a function of colony growth (worker production) and gyne production. The two outer columns represent the two extreme colony phenotypes; (1) common lineage queens that lack alternate-lineage sperm, cannot produce workers (green X), and consequently cannot produce gynes (red X), and (2) queens that lack same-lineage sperm, and have high worker production, but no intralineage gyne production (black X). Ant images are modified from Strassman and Queller (2008).**

larvae develop strictly according to their genotype (Helms Cahan et al. 2004). This genetic caste-determining mechanism necessitates a mutualism between two lineages that obligately co-occur in populations. We label these dependent lineage (DL) systems (Anderson et al. 2006a) because the continued survival of each lineage depends entirely on "worker-development" genes provided by its partner-lineage. The system can persist because queens mate with multiple males from each lineage. Interlineage crosses produce functionally sterile workers, intralineage crosses produce new fertile queens, and males are the product of unfertilized haploid queen eggs. Thus, the worker caste of every colony in the population is composed of F_1 interlineage hybrids, but both male and female reproductive forms remain "pure-breeding" lineages (Fig. 1).

The DL system is analogous to an obligate mutualism because the relative frequency of each interacting lineage can have a drastic effect on system dynamics, and the absence of either lineage will drive the system to extinction. Simulations based on a deterministic model suggest that both relative lineage frequencies and population-wide sex ratios may be subject to dramatic fluctuations, such that a DL system may persist only with the rapid convergence of lineage frequencies toward 0.5/0.5 (Yamauchi and Yamamura 2006). However, extensive field samples indicate that one lineage is often twice as frequent as its partner lineage (Helms Cahan et al. 2004; Anderson et al. 2006b; Schwander et al. 2006; Schwander et al. 2007a), creating an asymmetric selective regime that may be evident at two critical points of the life cycle (Fig. 1). The first is colony founding, during which the probability of early colony success increases with lineage rarity (negative frequency dependence; Helms Cahan et al. 2004; Anderson et al. 2006b; Schwander et al. 2006). This occurs because queens of the rare lineage mate randomly and primarily with males of the common lineage, acquire a high ratio of worker-to-gyne sperm, and avoid the energetic costs associated with rearing costly reproductives during early colony founding (Helms Cahan et al. 2004; Anderson et al. 2006b; Clark et al. 2006; Schwander et al. 2006; Volny et al. 2006). We predict that sperm ratios resulting from random mating will also be evident at reproduction; as a lineage becomes exceedingly rare, a large proportion of its queens will mate with few or no same-lineage males, and most or all females are genetically fated to develop as workers (Fig. 1). These rare lineage colonies (queens) have little to no fitness costs early in the life cycle, but at colony maturity will show limited gyne production or produce only haploid males. Although far fewer common

lineage colonies are expected to survive colony founding and attain reproductive maturity, those that do are predicted to possess ample gyne-destined sperm and produce both sexes. Hence, on average, gyne production at colony maturity should be positively frequency dependent; common lineage colonies should produce more gynes than rare lineage colonies.

As with all sexually reproducing organisms, mature colonies are under selection to produce sex ratios that confer maximal fitness benefits (Trivers and Hare 1976). Recent studies on DL *Pogonomyrmex* indicate that mating is random and queens cannot select the type of sperm (same or alternate-lineage) used to fertilize an egg (Helms Cahan et al. 2004; Anderson et al. 2006b; Clark et al. 2006; Schwander et al. 2006; Volny et al. 2006). Given these parameters, we assume that the queen does not influence the primary sex ratio and predict that sex ratios will vary in a deterministic fashion according to the relative frequency of each lineage in the population.

We examine sex ratio using both theoretical and empirical approaches. We model this system and generate predictions of how sex ratio will change with variation in lineage frequencies. To test model predictions, we first determine population lineage frequencies for 15 widely distributed DL populations. Next, we investigate sex ratio variation as a function of lineage frequency, and examine whether lower gyne production by the rare lineage drives overall sex ratio variation. We then investigate whether queens of the rare lineage compensate for low gyne production by increasing male production. Finally, we investigate the potential for resource availability to influence gyne production (Nonacs 1986) by analyzing the total reproductive biomass from two populations with very different lineage frequencies.

Materials and Methods

STUDY SYSTEM

On an annual cycle, DL *Pogonomyrmex* produce a cohort of males and reproductive females (gynes) that mate in synchronized and usually dense aggregations with as many as 10 males competing simultaneously for a single gyne (Hölldobler 1976). After mating with multiple males the gyne becomes a queen and establishes a new colony independently using only her metabolic storage reserves (Johnson 2000). Queen dispersal is relatively low and the colony-founding environment is occupied by a uniform distribution of mature colonies, resulting in $> 99\%$ mortality for newly mated queens (Gordon and Kulig 1996). Reproductive maturity is attained after the young colony has amassed a sufficient workforce, at about 3–5 years (Gordon 1995). Similar to most species of *Pogonomyrmex,* gynes are much larger and more expensive to produce than males (MacKay 1985; Smith and Tschinkel 2006). Maximal colony fitness requires balancing investment into growth and reproduction over the life of the colony (Munger 1992; Smith 2007), which may range from 15 to 43 years (Porter and Jorgensen 1988; Holldobler and Wilson 1990; Keeler 1993).

THE MODEL

To examine the effect of relative lineage frequency on sex ratio, we constructed both deterministic and stochastic simulation models including variables that affect colony growth, survival, and reproduction (see Appendix S1 for details). Two verbal models suggest that ecological differences between lineages could lead to the maintenance of stable but unbalanced lineage frequencies (Anderson et al. 2006b; Schwander 2006). These models propose that any ecological advantage gained by one lineage will be offset by the negative frequency-dependent advantage of colony founding success in the rare lineage. Here we explore the way in which an ecological advantage may influence relative lineage frequencies and the sex ratio of each lineage. We explicitly modeled differential colony growth because poor sperm ratios (excessive same-lineage sperm) impose sizeable costs on colony growth and survival, particularly during colony founding (Helms Cahan et al. 2004; Anderson et al. 2006b; Schwander et al. 2006).

A previous mathematical model of DL systems concludes that the rapid convergence of both male and queen genotypic frequencies toward 0.5 (of both interacting lineages) may be the sole explanation for DL system stability (Yamauchi and Yamamura 2006). However, observations on DL systems indicate that the lineage frequencies of most DL populations differ significantly from equal. Although many assumptions and parameters of our model are similar to those of Yamauchi and Yamamura (2006), ours differ by allowing one lineage an ecological advantage, and providing different parameters for colony establishment, growth, survival, reproductive potential, and the age of reproduction.

In our model, colony size, survival, and reproduction were determined by the genotype of the colony's queen (J1 or J2), the number and type of males she mated with (acquired sperm ratios assuming random and multiple mating), and colony age. The influence of sperm ratios on early colony survival, colony growth, and reproduction at maturity was the source of the frequency-dependent selection in our model that is characteristic of the DL mutualism (see Appendix S1 for details). Applying the binomial distribution, we modeled the proportional representation in the population of distinct classes of colonies defined by sperm ratio, age, and genotype. Each generation, established colonies survived with constant probability (i.e., 0.95), and according to colony size produced new males and gynes that then combined randomly to generate new colonies. Because colonies live for 15 or more years and are typically over-dispersed, new colonies are incorporated into the population in a density-dependent manner that reflects yearly colony mortality. All simulations were run using Mathematica 6 (Wolfram Research, Inc., Champaign, IL).

Table 1. Locations and lineage frequencies of dependent lineage populations (J1/J2) used to examine sex ratio. *N* = number of colonies used in each analysis, the *P* value under lineage frequencies is based on a sign test with a null hypothesis of equal lineage frequencies (0.5/0.5).

County in Arizona	Coordinates	Lineage frequencies		
		N	J1/J2	*P*
Cochise, MCR	N32°00′; W110°26′	72	0.48/0.52	ns
Gila, RYE	N34°03′; W111°22′	52	0.33/0.67	0.02
Maricopa QCR[1]	N33°15′; W111°58′	36	0.19/0.81	0.0004
Pima, SRT[1]	N31°57′; W110°57′	52	0.46/0.54	ns
Pima, SAS	N31°37′; W111°26′	48	0.25/0.75	0.0007
Pima, HWY	N31°47′; W110°41′	72	0.39/0.61	ns
Pima, MSR	N31°59′; W110°33′	46	0.61/0.39	ns
Pinal, CAB	N32°35′; W110°43′	48	0.56/0.44	ns
Santa Cruz, ELB	N31°39′; W111°05′	64	0.27/0.73	0.0002
Santa Cruz, MSL	N31°39′; W111°04′	68	0.29/0.71	0.0009
Santa Cruz, TMC	N31°34′; W111°03′	44	0.23/0.77	0.0004
Santa Cruz, PRV	N31°34′; W111°04′	50	0.28/0.72	0.003
Santa Cruz, AMA	N31°42′; W111°04′	73	0.33/0.67	0.005
Santa Cruz, PB1	N31°24′; W111°02′	41	0.37/0.63	ns
Yavapai, CTR	N34°42′; W111°52′	53	0.34/0.66	0.03

[1]Exhaustive sampling.

LINEAGE FREQUENCIES

We used a mean of 55 colonies per population to determined relative lineage frequencies (Table 1). Lineage was determined by analyzing diagnostic restriction fragments from a 650 bp portion of the Cytochrome Oxidase 1 (*cox*1) gene (Anderson et al. 2006a). DNA was extracted from one head per colony following the protocol outlined in Volny and Gordon (2002) and *cox*1 fragments were amplified using the primer pairs LCO/HCO according to methods of Anderson et al. (2006a). To confirm lineage, the PCR product was halved and reacted with two diagnostic restriction enzymes. Lineage J1 *cox*1 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. To confirm the restriction analyses, we sequenced two colonies of each lineage from all 15 populations for a total of 30 sequences. For each population, the null hypothesis of equal lineage frequencies (0.50/0.50) was examined with a sign test.

POPULATION SAMPLING

We sampled 15 J1/J2 populations across a range of elevations and habitats throughout central and southern Arizona in the southwestern United States (Table 1). The populations spanned 370 km of a roughly north to south transect, and the average distance between populations was approximately 130 km. All populations were separated by at least 2 km and population samples come from plots with an average radius of approximately 150 m.

COLONY SAMPLING

In DL *Pogonomyrmex*, exhaustive sampling is difficult because it requires extensive colony excavation. Many populations inhabit very rocky or hardpan soil and the underground chamber structure of a reproductively mature colony can attain depths exceeding 4 m. Conveniently, however, the upper one-half meter contains much of the colonies chamber area, (Whitford et al. 1976; Mackay 1981; Tschinkel 2004; Smith and Tschinkel 2006), and reproductive forms occupy the uppermost of these chambers on daily cycles prior to nuptial flights. Moreover, gynes and males can be lured to the top chambers by saturating the topsoil with water (simulating monsoon rains). Although exhaustive colony sampling provides a more accurate estimate of sex ratio, this is logistically impossible on a large scale. We only sampled exhaustively where the soil could be deeply excavated and where nearby roads provided access for our 250-L water tank.

Two of our 15 populations were selected a priori for exhaustive sampling (Table 1); one with relatively balanced lineage frequencies (0.46/0.54), and the other with extremely unbalanced lineage frequencies (0.19/0.81). The exhaustive sampling method consisted of pouring 4 L of water into the nest entrance, and then distributing an additional 16 L evenly within a 1-m radius around the nest entrance. The following morning, we carefully excavated the water-treated area to a depth of 25–50 cm, and collected all males and gynes. Two to three people working rapidly could excavate the upper chambers of 12–13 colonies per morning.

The remaining 13 populations were excavated using a relatively shallow method that systematically exposed horizontal nest

chambers that occur just 1–5 cm below the surface. We used a typical garden spade to expose 12 total areas (approximately 100 cm^2 each) around the colony entrance; four areas at 90° intervals in a 0.25-m radius, and eight areas at 45° intervals in a 0.5-m radius. Exposed sexual forms were placed quickly by hand in a white ceramic pail where sex was distinguished. Gynes and males were then tallied and returned to the nest. Within each population, colonies were sampled at random with respect to lineage. Absolute numbers of gynes and males are only compared between lineages within populations because a suite of variables including elevation, weather, time of day, and habitat exclude comparisons among populations across the sampled J1/J2 range.

SEX RATIO

We test three predictions of sex ratio and allocation using data from all 15 populations: (1) The sex ratios of each lineage will be similar when lineage frequencies are equal, but unequal lineage frequencies will result in the rare lineage producing a more male-biased sex ratio; (2) As lineage frequencies become increasingly unequal, the variation in sex ratio is explained by a decrease in rare lineage gyne production; and (3) If the rare lineage does not compensate for decreased gyne production with increased male production, it will invest less in overall sexual production. All predictions assume that mating and fertilization are random. Therefore, as a lineage becomes increasingly rare, its queens will acquire increasingly less intralineage (gyne-destined) sperm, and will produce fewer gynes than colonies (queens) from the common lineage (Fig. 1). However, male production should remain relatively unaffected by relative lineage frequencies because males are the result of unfertilized eggs. Prediction 3 stems from the hypothesis that sexual allocation decisions are made at a single time during the sexual production season, and consequently, rare lineage queens will produce fewer intralineage eggs during this time and cannot compensate for low gyne production by later increasing haploid male production; thus, the total amount of energy invested into sexuals will be lower in rare lineage colonies compared to those of the common lineage.

We test a fourth prediction using data from the two exhaustively sampled populations: (4) When intralineage sperm is at high frequency in the mating aggregation, more productive colonies will invest proportionally more resources into gyne production. We used *t*-tests to determine differences in total sexual production and sex-specific production between populations and lineages, and regression analysis to examine associations between total sexual production and gyne production within each lineage.

We present the sex ratio for each lineage in all 15 populations (30 lineages) as the total number of males divided by the total number of males + gynes. We examine the change in sex ratio as a function of lineage frequency using regression analysis (prediction 1). We determine whether changes in male or gyne production drive changes in the sex ratio (prediction 2) by comparing the contribution of the rare lineage to overall male and gyne numbers across all populations (also as a function of lineage frequency); this analysis was performed only on the rare lineage because the lineages are not independent (the frequency of the common lineage equals one minus the frequency of the rare lineage). Male and gyne slopes were compared using a *t*-test. For predictions 3 and 4, we first converted the total numbers of males and gynes produced to an investment parameter ($biomass^{0.7}$) to account for the large sexual size dimorphism in this species. We used the average dry mass of J1/J2 lineage reproductives reported in the literature (male: 8.6 mg, gyne: 26.4 mg, Wagner and Gordon 1999) and multiplied this by the numbers of each sex produced, per colony, lineage, and population. We applied a 0.7 power correction to biomass to account for sex-specific differences in production and maintenance costs (e.g., respiration) of males and gynes (Boomsma 1989). We tested whether male, gyne, and total reproduction was different between lineages on a per colony basis using paired *t*-tests (paired by population).

Results

THE MODEL

We developed a model to predict conditions under which the rare lineage should produce less gynes resulting in a more male-biased sex ratio. Based on laboratory and field data, we incorporated realistic parameter values for life-history traits of *Pogonomyrmex* into the model (Appendix S1). Similar to the majority of parameter sets in Yamauchi and Yamamura (2006), our simulations show that the system is quickly driven to extinction within 200 generations. However, our simulations revealed that when one lineage has an ecological advantage, the system can be stable across a range of relative lineage frequencies. When one lineage is provided with an ecological advantage the simulation shows that male bias in the rare lineage increases as a function of increasing lineage skew, whereas male bias in the common lineage decreases (Fig. 2). The slopes differ significantly between the lineages ($t_{398} = 46.9$, $P < 0.0001$, common lineage; slope $= 0.26$, $R^2 = 0.88$, rare lineage; slope $= -0.31$, $R^2 = 0.81$). The amount of change in male bias across variation in lineage frequencies (slopes in Fig. 2) is approximately 25% greater in the rare as compared to the common lineage.

LINEAGE FREQUENCIES

Sequences from the *cox*1 gene confirmed the results of the restriction analysis and revealed that all 15 sampled populations were composed of lineage J1 and J2. According to sign tests, the lineage frequencies of nine populations differed significantly from equal. Lineage J1 was at lesser frequency in 13 of our 15 sampled populations (Table 1). Pooling colonies from all 15 populations,

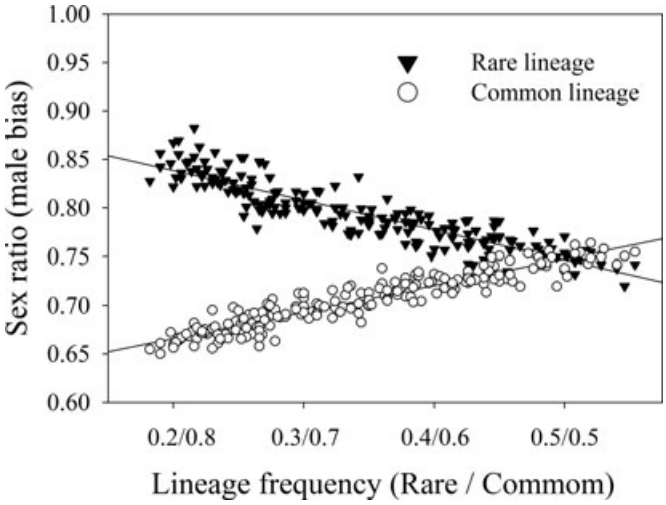

Figure 2. **A stochastic simulation predicting the numerical sex ratio (male bias) of both rare and common lineages across a range of relative lineage frequencies. The common lineage was provided with a 15% ecological advantage in colony growth. The simulation ran 200 generations. Sex ratios are male biased when lineage frequencies are 0.5/0.5, because male production is a function of colony size, but gyne production relies on both colony size and lineage frequency. When lineage frequencies are 0.5/0.5, only half of the matings on average will be with the same-lineage, decreasing the amount of sperm destined for gyne production.**

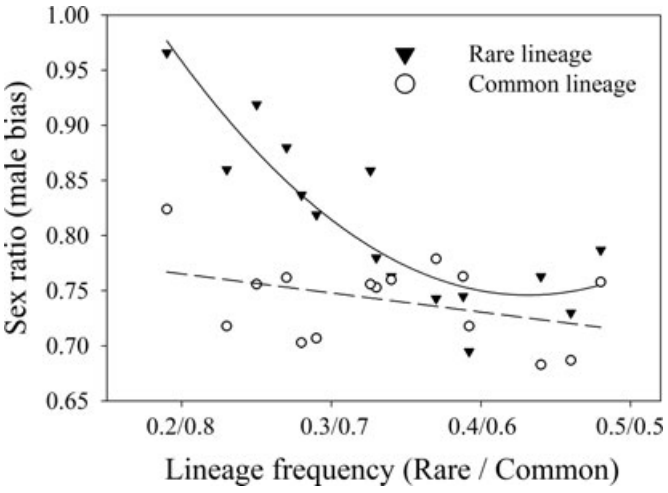

Figure 3. **Lineage-specific fitness expressed as numerical male bias in the sex ratio regressed on relative lineage frequency. A quadratic polynomial was fitted to the rare lineage (see text). Male bias in the rare lineage was strongly correlated with relative lineage frequency (Adj $R^2 = 0.78$, $F_{2,12} = 25.4$, $P < 0.0001$). Male bias in the common lineage was unassociated with relative lineage frequencies (Adj $R^2 = 0.09$; $F_{1,13} = 2.3$; $P = 0.15$).**

the mean lineage frequency of J1 is significantly less than 0.5 (Sign test, $P < 0.0001$; $n = 694$ colonies; mean $\pm$ SD: 0.336 ± 0.09; 95% CI $\pm$ 0.034).

SEX RATIO

The results are consistent with our first prediction; that male-biased sex ratios increase in the rare lineage as lineage frequencies become increasingly unequal. The sex ratio of the rare lineage was significantly and negatively correlated with relative lineage frequency (Adj $R^2 = 0.64$; $F_{1,13} = 25.8$; $P = 0.0002$, Power = 98%, alpha = 0.05). This linear fit was improved significantly ($F_{1,12} = 5.25$, $P < 0.05$) by the addition of a quadratic term (Fig. 3), yielding a second degree polynomial of the form; [$\hat{Y} = 1.5 - 3.4x + 3.9x^2$]. The fitted quadratic regression explained 14% more variation in male bias with greater power: (Adj $R^2 = 0.78$, $F_{2,12} = 25.4$, $P < 0.0001$, Power = 99%, alpha = 0.05). On the other hand, the sex ratio of the common lineage was unassociated with relative lineage frequencies (Fig. 3, Adj $R^2 = 0.09$; $F_{1,13} = 2.3$; $P = 0.15$).

Our second prediction was also supported, as the increased male bias in the rare lineage sex ratio stems from a decrease in gyne production more than an increase in male production. The contribution of the rare lineage to a population's males and gynes is significantly predicted by the frequency of the rare lineage (Males: slope = 0.75, Adj $R^2 = 0.61$, $F_{1,13} = 22.9$, $P < 0.001$; Gynes: slope = 1.28, $R^2 = 0.81$, $F_{1,13} = 59.4$, $P < 0.0001$), but gyne production increases much faster than male production as lineage frequencies approach equal (difference between slopes: $t = 2.32$, df = 28, $P < 0.05$).

Our third prediction, that the rare lineage invests less in overall sexual production, is also supported. However, the trend suggests that rare lineage colonies do not greatly increase male production in a compensatory manner. In fact, common lineage colonies, on average, produced more males than rare lineage colonies, suggesting that overall production of sexuals may be lower in rare lineage colonies independent of frequency-dependent effects. In terms of per colony investment in sexual biomass, the rare lineage invested less in gynes than the common lineage ($t = 2.9$, df = 14, $P < 0.01$), but similarly in male production ($t = 2.0$, df = 14, $P = 0.07$), which led to significantly decreased sexual production in the rare lineage ($t = 2.3$, df = 14, $P = 0.03$).

Our fourth prediction that more productive colonies would invest proportionally more resources into gyne production was strongly supported by the results of the deep excavations. Only the rare lineage (J1) from the population with unequal lineage frequencies (QCR) did not show a significant positive correlation between total reproductive biomass and gyne investment (Fig. 4, SRT lineage J1; Adj $R^2 = 0.6$, $F_{1,16} = 26.2$, $P < 0.0001$, SRT lineage J2; Adj $R^2 = 0.87$, $F_{1,19} = 132.8$, $P < 0.0001$, QCR lineage J1; Adj $R^2 = 0.07$, $F = 0.57$, $P < 0.61$, QCR lineage J2; Adj $R^2 = 0.57$, $F = 38.2$, $P < 0.0001$). In the SRT population, the proportion of total reproductive biomass invested in gynes

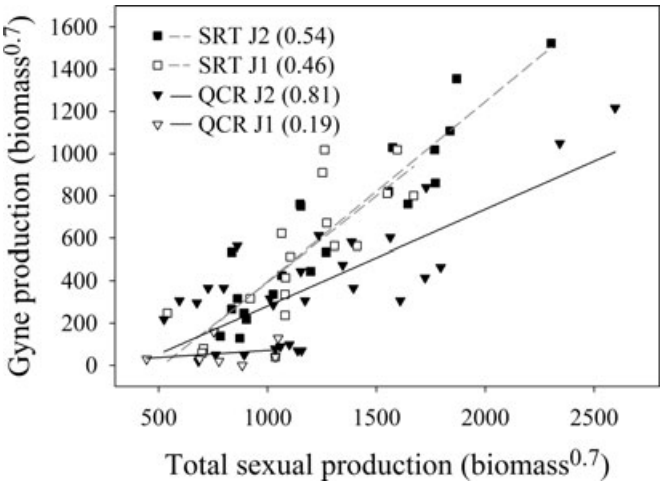

Figure 4. Total gyne production by each lineage in the deeply excavated populations graphed as a function of total sexual production. Each symbol represents a colony. Only the slopes of the SRT lineage pair do not differ significantly (see Results).

was similar between lineage J1 and J2 ($t = 0.31$, df $= 37$, $P < 0.76$). In the QCR population however, this proportion differed significantly by lineage (Table 2, $t = 3.91$, df $= 34$, $P < 0.0004$).

Total reproductive biomass was similar between the exhaustively sampled populations SRT and QCR ($t = -0.94$, df $= 73$, $P = 0.35$), but both gyne and male biomass differed significantly. This effect was caused by the population with very unequal lineage frequencies (QCR) producing both a significant deficiency of gynes ($t = 3.5$, df $= 73$, $P = 0.0008$) and a significant surplus of males ($t = -3.0$, df $= 73$, $P = 0.003$) relative to the balanced SRT population (Table 2).

Discussion

Ant colonies of DL *Pogonomyrmex* experience life-history stages that are characterized by the seasonal production of different castes, and colony fitness is greatly enhanced by efficient caste production at different stages in the colonies life cycle (Oster and Wilson 1978). Gyne production during colony growth stages or excessive worker production during the brief reproductive stage will decrease colony fitness. Because a typical ant colony often devotes less than 10% of its lifetime production to reproductive forms (Bourke and Franks 1995), colony fitness in a DL system relies strongly on the queen acquiring the proper proportion of worker/gyne-destined sperm from males of each lineage. Previous studies have demonstrated frequency-dependent selection during early colony establishment (Helms Cahan et al. 2004; Anderson et al. 2006b; Schwander et al. 2006). Our results show that the final sex ratio of mature colonies is also associated with established lineage frequencies. Thus, the effects of frequency-dependent selection are evident at two points in the life cycle; colony founding success of the rare lineage is negatively frequency dependent, and gyne production by the common lineage is positively frequency dependent (Fig. 1). Common lineage queens are capable of producing relatively more reproductive females, and potentially greater overall sexual investment. Combined with previous findings for colony establishment, our results suggest that the general efficiency of diploid caste production in a DL system is strongly influenced by sperm ratios generated from random mating and relative lineage frequencies.

Existing molecular data also provides strong support for our sperm ratio hypothesis. Three separate studies on the H1/H2 lineage pair demonstrate that interlineage queens are produced only in small numbers, and only when no intralineage offspring are available for rearing (Helms Cahan et al. 2002; Helms Cahan and Keller 2003; Schwander et al. 2007b). That is, when the colonies queen did not acquire intralineage (gyne-destined) sperm because it was at low frequency in the mating aggregation. Schwander et al. (2007b) found a significant correlation between increasingly unequal lineage frequencies and the proportion of colonies producing only interlineage gynes. Although these studies

Table 2. Characteristics of the two exhaustively sampled populations QCR and SRT.

Population	Frequency[2]				Numbers[3]		Biomass Mean ± SE[4]	
	N[1]	Lineage	Male	Gyne	Males	Gynes	Gynes	Total
QCR								
Lineage J1	7	0.19	0.18	0.04	1159	41	58 ± 23	805 ± 79
Lineage J2	29	0.81	0.82	0.96	5358	1105	377 ± 55	1210 ± 92
SRT								
Lineage J1	18	0.46	0.46	0.40	2531	933	513 ± 75	1147 ± 73
Lineage J2	21	0.54	0.54	0.60	3014	1373	647 ± 87	1294 ± 96

[1]Colony sample size.

[2]The frequency of each lineage in the population, and the proportion of males and gynes produced by each lineage in the population.

[3]Absolute numbers of each sex produced by each lineage in the population.

[4] Mean colony reproductive biomass$^{0.7}$ expressed as dry weight in milligrams.

highlight the extreme colony phenotype (colonies that completely lack intralineage sperm; see Fig. 1), they provide strong support that the decrease in rare lineage gyne production documented by this study (Fig. 3) is due primarily to the lack of gyne-destined sperm available in the mating aggregation.

The results of an earlier mathematical model suggest that offsetting frequency-dependent selection pressures result in the oscillation of relative lineage frequencies around 0.5/0.5 (Yamauchi and Yamamura 2006). Under the majority of parameter sets these oscillations grow in amplitude each subsequent generation causing the rapid extinction of the DL system. Under a few rather narrow parameter sets however, the oscillations dampen within a few generations, rapidly converging on 0.5/0.5. This occurs because positive and negative frequency-dependent selection pressures have been equalized; the advantage of an increased increment of gynes in one lineage is equal to the advantage of an increased increment of workers in the other lineage (Yamauchi and Yamamura 2006). This theoretical explanation is satisfying because when lineage frequencies converge on 0.5/0.5 both lineages are freed from the effects of frequency-dependent selection, and the system as a whole may experience the lowest selection pressure. However, our empirical results show that the two lineages most often coexist at frequencies that differ significantly from equal (Table 1). Across 21 known populations of the J1/J2 lineage pair (15 analyzed here, plus six from Schwander et al. 2007a), the mean relative lineage frequency (0.34/0.66) is highly inconsistent with the rapid convergence of lineage frequencies toward 0.5/0.5. Moreover, in 19 of 21 populations, lineage J2 is the most frequent lineage suggesting that it may possess an ecological advantage over lineage J1 that operates independent of frequency-dependent effects. The inconsistency between the Yamauchi and Yamamura (2006) model and the empirical data presented here highlights a key component of system persistence: How are system dynamics affected if one lineage has an ecological advantage over its partner lineage? And given an ecological advantage, at what relative lineage frequency does the benefit of increased worker production in the rare lineage balance increased gyne production in the common lineage?

THE MODEL

Our simulations provided up to a 50% ecological advantage to one lineage and modeled colony growth, reproduction, and survival (Appendix S1). The lineage given the advantage became common and showed increased overall worker productivity, increased survival rate of immature colonies, and increased gyne production in established colonies. This added advantage stabilized the unequal lineage frequencies by offsetting the extreme cost paid by the common lineage during early colony survival. Thus, when one lineage has an ecological advantage, our simulation model indicates that this compensating factor allows the system to be stable across a broad range of relative lineage frequencies. Under these conditions, the simulation shows that male bias in the rare lineage increases as lineage frequencies become increasingly unequal, a result highly consistent with our empirical data (Figs. 2 and 3). Empirical results from both shallow and deep sampling confirm that male bias in the rare lineage is due to gyne production decreasing steeply with lineage rarity whereas male production remains relatively unaffected. As first predicted by the model of Yamauchi and Yamamura (2006), the variation in male production among colonies is much smaller than that of female production. This is also a prediction of our model, and is intuitive because males result from unfertilized eggs, so their production is less reliant on acquired sperm ratios. Given that empirical male production across the range of sampled lineage frequencies was similar between the lineages, it appears that male production was relatively unaffected by the ecological advantage provided to lineage J2. The dynamics of gyne production are far more relevant, because unlike male production, this parameter relies heavily on sperm ratios, random mating, and mating frequency. Our model also predicts that gyne production in the common lineage should increase as lineage frequencies become unequal (Fig. 2), but empirical results show that gyne production remained relatively constant across a broad range of lineage frequencies (Fig. 3). This suggests that most common lineage colonies possess enough gyne-destined sperm to often realize an upper limit on gyne production.

The interaction between positive and negative frequency-dependent selection is complex because reproductive investment increases with worker number in *Pogonomyrmex*, and worker production is a prerequisite for gyne production (Mackay 1981; Cole and Wiernasz 2000; Smith and Tschinkel 2006; Smith 2007). Because sterile workers may represent over 90% of a colony's lifetime investment, it follows that queens acquiring worker-biased sperm ratios in addition to some lesser quantity of gyne-destined sperm will have the highest reproductive success. Thus, the greatest fitness returns may be realized across a range of unequal lineage frequencies wherein the common lineage has an ecological advantage and shows relatively greater gyne production, and this benefit is counterbalanced in the rare lineage through an increase in early colony survival and colony growth. Across this hypothetical range of relative lineage frequencies, queens from both the rare and common lineage can survive the selective pressures of colony founding, colonies can grow quickly, and queens still possess sufficient gyne-destined sperm at colony maturity. Based on our empirical results, we speculate that this range may exist where sex ratios between the rare and common lineage are most similar (0.33/0.67 – 0.40/0.60; Fig. 3). The significant polynomial relationship (Fig. 3) supports this argument and suggests that within this range the ecological advantage of lineage J2 actually results in reproductive benefits to the rare lineage (J1) in

the form of increased gyne production. At some point however, the advantage of rarity will produce diminishing fitness returns because the chances become remote that a queen from a very rare lineage (0.1) will acquire intralineage sperm. Thus, severely reduced gyne production in the rare lineage is a likely precursor of localized system extinction, but may only occur when the availability of same-lineage sperm drops below a certain threshold.

SEX ALLOCATION

Because diploid eggs lack phenotypic plasticity for caste development, colonies in DL systems may have difficulties regulating colony growth and sexual production as compared to environmental caste-determining species. However, DL queens can influence gyne production via a maternal effect triggered by overwintering (Schwander 2008). But the DL queen has only partial control over the primary sex ratio, because she determines whether an egg is fertilized but apparently not the type (lineage) of sperm used in fertilization (Helms Cahan et al. 2004; Anderson et al. 2006b; Clark et al. 2006; Schwander et al. 2006; Volny et al. 2006). Limitations on the queens' ability to produce gynes during the seasonal reproductive phase are probably caused by both the duration of the overwintering effect on the producing queen, and a metabolic upper limit on her ability to generate eggs. It is unclear what happens to diploid worker-destined brood produced during the brief reproductive phase, but it's possible that the maternal effect on caste determination may preclude their development. Small numbers of worker pupae are sometimes found developing alongside the sexual pupae, but in many colonies, developing workers are completely absent, suggesting that some interlineage genotypes may abort as a result of developmental failure or be culled by workers during early development (K. E. Anderson, unpubl. data).

Our results provide support for the hypothesis that unequal lineage frequencies result in reduced gyne production by the rare lineage, but an ecological advantage or disadvantage held by either lineage generates a similar prediction. That is, the degree to which relative lineage frequencies are unequal in a given population may reflect either the superiority of the common lineage, or inferiority of the rare lineage to gather and utilize resources, which are then invested in gynes. These two hypotheses differ in their predicted mechanisms, whether resource acquisition or worker-gyne sperm ratios constrain gyne production (Nonacs 1986). Although our study was not designed to distinguish these hypotheses, it is likely that both processes influence sex allocation in different ways. Resource availability may drive sex allocation when lineage frequencies are relatively balanced, but worker–gyne sperm ratios may exert a greater influence as lineage frequencies become increasingly unbalanced. Results from the two exhaustively sampled populations provide some support for this idea. Sexual allocation by three of the four lineages was consistent with the resource availability hypothesis. Only the rare lineage (J1) from the population with very unequal lineage frequencies (QCR) did not show a significant positive correlation between total sexual biomass and gyne biomass (Fig. 4). However, if lineage frequencies simply reflect the degree of ecological advantage, we might predict that an extremely common J2 lineage (0.81) would show greater gyne production than a slightly common (0.54) J2 lineage. But gyne production was significantly lower in the very common (0.81) lineage ($t = 2.75$, df $= 48$, $P < 0.008$), a result better explained by the sperm ratio hypothesis: The relative surplus of common-lineage sperm at the mating flight results in a large majority of common lineage queens dying prior to reproduction due to the lack of worker-destined sperm needed to survive colony founding (Helms Cahan et al. 2004; Anderson et al. 2006b; Schwander et al. 2006). As a direct result, the variation in common lineage sperm ratios is reduced to reflect the rather small subset of queens acquiring enough worker sperm to attain reproductive size. Many of these colonies likely contain a surplus of gyne-destined sperm such that sex allocation may be constrained by the efficiency of worker production which should vary across this subset in accordance with the strength of selection on colony founding and growth. The significantly different slopes between J2 lineages (Fig. 4) suggest that this efficiency may be influenced by relative lineage frequency and to some extent may explain the proportion (0.28) of common lineage colonies with extremely low gyne production in the very unbalanced QCR population. In general however, the finding that gyne production is a function of total reproductive output in all but the extremely rare lineage (Fig. 2) suggests that when gyne-destined sperm is not limited, the effect of lineage frequency on sex allocation is greatly reduced, and colonies may then invest in reproduction according to resource availability.

Conclusion

DL populations are abundant and widespread throughout the southwestern United States (Anderson et al. 2006a; Schwander et al. 2007b; Anderson et al. 2008) suggesting that the system responds well to ecological change and that conditions for system persistence are not as narrow as first thought. Our results show that the DL reproductive mode generates lineage-specific sex ratios that become evident as lineage frequencies become increasingly unequal. In contrast to the organisms and assumptions used to construct early theories of sex allocation (Fisher 1958; Trivers and Willard 1973) the ant colonies in this study are similar to birds and mammals (Frank 1990) in that they are characterized by extensive parental (colony) care, a genetic determination mechanism that constrains sex ratio, potentially complex mechanisms for adjusting investment in the sexes, and fitness trade-offs between current sex ratio and future reproduction.

The unique structure of DL populations provides a system to test the effects of both negative and positive frequency-dependent selection pressures on the stability of lineage interdependence with dynamics very similar to obligate mutualisms. As seen with dispersal mutualisms (Holland and DeAngelis 2001), DL dynamics are strongly influenced by the relative number of individuals present in each of the interacting populations, highlighting the importance of frequency-dependent selection in models of mutualistic coevolution (Levin and Udovic 1977). Thus, DL systems can serve as a model to investigate coevolutionary questions including the stability of lineage interactions, how interactions vary across time and geographic space, and how the interacting genomes adapt to their environment and one another (Bull and Rice 1991; Travis 1996; Gomulkiewicz et al. 2003; Nuismer et al. 2003).

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Deterministic and stochastic simulation models of a dependent lineage system.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).

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