Genetic polyethism in leaf-cutting ants

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Females mating with multiple males (polyandry) is taxonomically widespread but is hard to explain in many animals in which it has no obvious direct benefits. In some, of which social insects are the best example, it is suggested that females benefit from the increased genetic diversity of their offspring. Social insect colonies express division of labor, and if genotypes behave differently (genetic polyethism), then genetically diverse colonies may be fitter. However, unequivocal evidence of genetic polyethism is only known from honey bees. Here, we show that such a genetic influence on behavior is also present in the leaf-cutting ant *Acromyrmex echinatior*. In 2 of the 3 colonies examined, we found that the offspring of some fathers (patrilines) were more likely to engage in waste management, whereas other patrilines were more likely to engage in foraging. When we increased the stimulus for waste management, the representation of patrilines in the ants that responded was the same as normally engaged in waste management in 2 colonies but differed in the third colony. The leaf-cutting ant *A. echinatior* therefore shows genetic polyethism. Although other factors such as disease resistance may have also played a role in the evolution of polyandry, the results suggest that genetic polyethism may be widespread in social insects and potentially of general importance in the evolution of polyandry. *Key words:* division of labor, genetic diversity, polyandry, response threshold, social insect. *[Behav Ecol 21:1165–1169 (2010)]*

Although multiple mating by females with different males (polyandry) is a common and phylogenetically widespread behavior, it is often hard to explain. This is because polyandry is generally associated with significant costs, such as energy expended, increased predation risk while locating and copulating with mates, and increased exposure to sexually transmitted diseases, yet clear benefits can be difficult to identify. In some species, the behavior has obvious direct benefits (e.g., sperm, nuptial gifts, or paternal care) but more subtle indirect genetic benefits (e.g., trading up to a male of better genetic quality or diluting genetically incompatible matings that may produce nonviable offspring) may explain the behavior in many other species (Arnqvist and Nilsson 2000; Jennions and Petrie 2000). Polyandry increases the genetic diversity of offspring produced, and one hypothesized benefit is that this increases the probability of a female producing at least some offspring with the appropriate level of a genotypically variable trait for unpredictable environment conditions (bethedging; Watson 1991; Yasui 1998). However, evidence for a benefit from offspring genetic diversity in most animals is lacking (Jennions and Petrie 2000).

Social insects provide the best example of polyandry providing a benefit due to offspring genetic diversity. Polyandry is a derived trait in social insects, with only a quarter of species being polyandrous and only 14 genera having so far been shown to exhibit polyandry to a significant extent (Hughes, Oldroyd, et al. 2008; Hughes, Ratnieks, et al. 2008). Mating with multiple males carries significant costs to social insect females (e.g., Baer et al. 2006), although direct benefits of the behavior, such as nuptial gifts, are not present, and the brief anonymous nature of mating interactions in most social insects makes trading up impossible (Crozier and Fjerdingstad 2001). Explanations for the evolution of polyandry in social insects have therefore centered on genetic benefits. There are 3 leading hypotheses, including that polyandry di-

lutes genetically incompatible matings which may produce nonviable offspring or sterile diploid males (Page 1980; Tarpy and Page 2002) and produces genetically diverse offspring colonies which are more resistant to disease (Baer and Schmid-Hempel 1999; Tarpy 2003; Hughes and Boomsma 2004, 2006; Seeley and Tarpy 2007; Reber et al. 2008; Hughes et al. 2010). The third of these 3 leading hypotheses for the evolution of polyandry in social insects is that workers differ genotypically in their propensities to engage in particular tasks, which then allows the division of labor of genetically diverse colonies to respond more appropriately to changing conditions (reviewed by Oldroyd and Fewell 2007). Such genetic influences on behavior have been abundantly demonstrated in the honey bees *Apis mellifera* and *A. florea*, in which they are due to patriline differences in response thresholds for task-related stimuli (e.g., Robinson and Page 1988; Page et al. 1989; Oldroyd et al. 1994; Kryger et al. 2000; Jones et al. 2004, 2007; Chapman et al. 2007). However, evidence from other social insects is far more limited. Several studies with ants and wasps have found that individuals from different colonies or matrilines differ in behavior (Stuart and Page 1991; Snyder 1992; O'Donnell 1996, 1998; Julian and Fewell 2004), but such differences can be due to maternal effects or rearing conditions, as well as genetic effects. Patriline differences, which provide conclusive evidence of genetic effects, have been found for morphological worker caste in several ants, with individuals differing according to their patriline in their probabilities of developing into the different worker castes (Hughes et al. 2003; Rheindt et al. 2005; Hughes and Boomsma 2007, 2008; Jaffé et al. 2007). However, similar patriline effects have not been demonstrated for behavioral tasks in any social insect other than honey bees.

Here, we examine whether patriline influences behavioral task in the polyandrous leaf-cutting ant *Acromyrmex echinatior*. We focus on the external tasks of foraging and waste management, which workers engage in toward the end of their lives (Hart and Ratnieks 2002; Waddington and Hughes 2010). We also experimentally increase the stimulus for waste management to determine whether the representation of patrilines in the ants responding to this is different to that of ants originally engaged in waste management,

which could suggest patriline differences in response threshold.

MATERIALS AND METHODS

Three monogynous colonies of *A. echinatior* (Ae213, Ae216, and Ae312) were used which had been collected in Gamboa, Panama, between 2003 and 2006. Colonies were kept in plastic boxes (17 × 36 × 54 cm), with fungus gardens contained within inverted plastic beakers and a 10-cm diameter pot in which the ants deposited waste. Colonies were maintained on a diet of privet leaves (*Ligustrum* spp.) at 80 ± 5% relative humidity and 26 ± 2 °C.

Sampling

We first marked the workers engaged in waste management or foraging with task-specific colors over the course of 3 days. Ants seen engaged in these tasks were removed using forceps, cooled on ice, and marked with paint. They were kept individually for 5 min to confirm the paint marking had not adversely affected them and then replaced in their colony. This procedure did not affect their subsequent behavior (Waddington and Hughes 2010). We collected and marked workers for 8 h per day for 3 days. We then added 5 ml of waste particles, taken from the waste pile of the same colony, to an area around the nest entrance so that it would be encountered not only by waste management workers but also by foragers and the internal work force. Over the following 6 h, we paint marked all the workers that transported the added waste to the waste pile, using the same procedure as before but with a new color. After this period, we then collected paint-marked individuals for genotyping: 1) We collected workers that had been originally engaged in foraging or originally engaged in waste management to determine if genotype influenced this allocation. 2) We also collected internal workers from the fungus garden. These included inactive foragers and waste management workers, as well as workers engaged in other tasks, and were thus representative of the general worker population. We compared whether the representation of patrilines in waste management workers and foragers differed from these internal workers. 3) We collected individuals that had responded to the additional waste stimulus as well in order to determine if their patriline representation differed from that of the original waste management workers. In all cases, individuals sampled were selected to be of similar size and age, as inferred from their cuticular coloration (Armitage and Boomsma 2010).

Molecular and statistical analyses

DNA was extracted from ant legs using 5% Chelex (Bio Rad, Hercules, CA) and amplified at microsatellite loci Ech3385, Ech4126, Ech4225, and Atco15 (Ortius-Lechner et al. 2000; Helmkampf et al. 2008). Polymerase chain reaction (PCR) amplification was performed using 10 μl mixtures containing 40 μM deoxyribonucleotide triphosphates, 3 mM MgCl$_2$, 0.5 μM primers, 0.5 U of *Taq* DNA polymerase, 1× buffer, and 1 μl DNA. Amplifications were run in GeneAmp 9700 PCR Systems with an initial denaturing step of 94 °C for 2 min followed by 35 cycles of 94 °C for 2 min, 55 °C (Ech3385, Ech4225, and Atco15) or 60 °C (Ech4126) for 45 s, and 72 °C for 2 min, and finally 72 °C for 7 min. Products were genotyped using an ABI 3130*xl* capillary sequencer, and allele sizes determined by comparison with internal size standards. The genotypes of the mother queen and her multiple mates were deduced from the multilocus worker genotypes and individuals assigned to patrilines. Individuals which could not be assigned to patri-

lines due to failed PCR amplification or sharing the same genotype at a diagnostic locus as a heterozygous mother queen were excluded from the analysis. The total numbers of individuals genotyped successfully that were, respectively, waste management workers, foragers, internal workers, or workers responding to the increase in waste were 21, 21, 21, and 19 for colony Ae213, 34, 44, 31, and 25 for Ae216, and 43, 61, 43, and 27 for Ae312. The frequencies of individuals in each patriline that were waste management workers, foragers, or internal workers, or which responded to the increase in stimulus for waste management, were compared using Fisher's Exact tests. The effect sizes of the patriline differences were estimated using Cramer's *V* which measured the strength of the association (from 0 = no association to 1 = maximum association; (Grissom and Kim 2005) between patriline and the frequencies of individuals that were 1) waste management workers and foragers or 2) waste management workers or workers responding to the increase in waste. We also calculated Cramer's *V* for similar data obtained in honey bees by Robinson and Page (1988).

RESULTS

Colonies Ae213, Ae216, and Ae312 contained 7, 6, and 5 patrilines respectively. There was considerable paternity skew, and the representation of patrilines varied markedly between waste management workers, foragers, internal workers, and workers that responded to the increase in waste stimulus (Figure 1). The frequency distribution for the patrilines of workers engaged in waste management differed significantly from that of internal workers in colonies Ae213 ($P = 0.025$) and Ae312 ($P = 0.045$) but not Ae216 ($P = 0.12$). Patrilines within colonies Ae213 ($P = 0.041$) and Ae312 ($P = 0.02$) differed significantly in their propensity to engage in waste management or foraging (Figure 2) but did not differ in colony Ae216 (Fisher's $P = 0.307$). The effect sizes (Cramer's *V*) of patriline on whether an individual engaged in waste management or foraging were 0.532, 0.247, and 0.314 for colonies Ae213, Ae216, and Ae312, respectively (mean ± standard error [SE]: 0.364 ± 0.086). This compares with very similar effect sizes of between 0.13 and 0.67 (0.33 ± 0.06) for undertaking by honey bees in the study by Robinson and Page (1988), and somewhat larger effect sizes for guarding in honey bees of between 0.1 and 0.83 (0.48 ± 0.07).

The representation of patrilines in the individuals that aided in the removal of the experimentally increased waste did not differ from the original waste management workers in 2 colonies (Ae216: $P = 0.911$; Ae312: $P = 0.852$; Figure 3). There were, however, significant differences in colony Ae213 ($P = 0.016$), with patriline 7 and to a lesser extent patrilines 1, 4, and 6, appearing more likely to engage in waste management when the stimulus was increased. The Cramer's *V* effect sizes for patriline in response to the increased waste were 0.572, 0.091, and 0.16 for colonies Ae213, Ae216, and Ae312, respectively (mean ± SE: 0.274 ± 0.15).

DISCUSSION

Although our sample sizes were relatively small, we found that patrilines differed significantly in their representation in foragers and waste management workers in 2 of the 3 colonies we examined. In at least 2 colonies, therefore, there was a significant genetic influence on the propensities of individuals to engage in these 2 tasks. Similar patriline effects have been found on caste determination in *A. echinatior* (Hughes et al. 2003; Hughes and Boomsma 2007), as well as other ants (Rheindt et al. 2005; Jaffé et al. 2007) and have been abundantly demonstrated for behavioral tasks in honey bees (Robinson and Page 1988; Page et al. 1989; Oldroyd et al. 1994; Kryger et al. 2000;
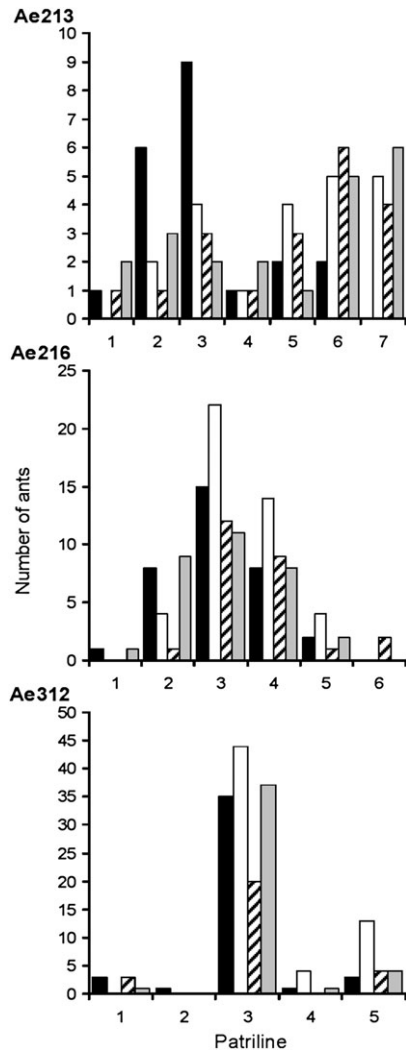
Figure 1
Frequencies of individuals sampled per patriline from colonies Ae213, Ae216, and Ae312 that were original waste management workers (black), original foragers (white), internal workers (dashed), or which responded to engage in waste management following an experimental increase in stimulus (gray).
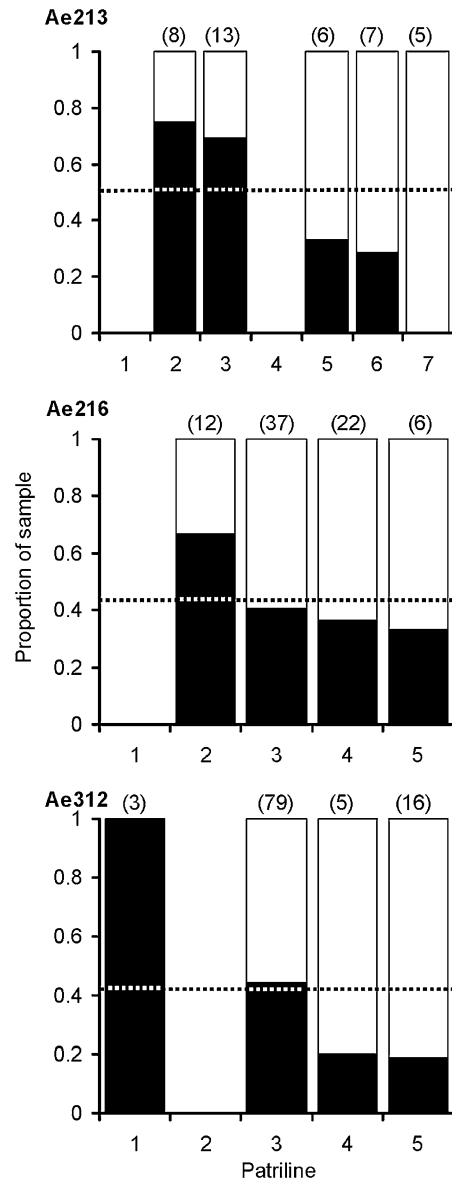
Jones et al. 2004, 2007; Chapman et al. 2007; Oldroyd and Fewell 2007). The results suggest that whether an *A. echinatior* worker engages in waste management or foraging is influenced by its genotype, although other factors will also be important. The effect sizes for the patriline influence were very similar to those found for undertaking in honey bees (Robinson and Page 1988), suggesting that genetic polyethism may be just as strong in *A. echinatior* as in honey bees. The patriline effect on guarding is greater in honey bees (Robinson and Page 1988), and it will be interesting to see if similar task-specific differences in the magnitude of genetic polyethism are present in *A. echinatior*.

Increases in stimuli for a particular task within honey bee colonies result in more workers engaging in the task (Fewell and Bertram 1999; Breed et al. 2002), and this is also true for waste management in leaf-cutting ants (Waddington and Hughes 2010). In the case of honey bees, this response is influenced by genotype, with the representation of patrilines differing between workers originally engaged in a task and those responding to an increased stimulus (Jones et al. 2004; Chapman et al. 2007). This is thought to be because patrilines have

Figure 2
Proportions of individuals sampled per patriline from colonies Ae213, Ae216, and Ae312 that were original waste management workers (black) or original foragers (white). The dashed lines show the expected ratio given the numbers of individuals genotyped if patriline does not influence an individual's propensity to become a forager or waste management worker (lines differ slightly from 0.5 because not all individuals could be assigned to patrilines). Sample sizes are given in parentheses above the columns for each patriline. Rare patrilines with less than 3 workers have been excluded.

different response thresholds and thus an increase in stimulus results in the response thresholds of more patrilines being met (Oldroyd and Fewell 2007; Oldroyd and Thompson 2007). When waste levels were increased in the current study, the representation of patrilines was similar to those originally engaged in waste management in 2 of the colonies but differed in colony Ae213. This could potentially be explained by the response thresholds of patrilines differing relatively more in the former 2 colonies than in Ae213, although experiments directly measuring response thresholds would be needed to confirm if this is the mechanism. Similar changes in patriline representation in *A. echinatior* occur in response to changes in
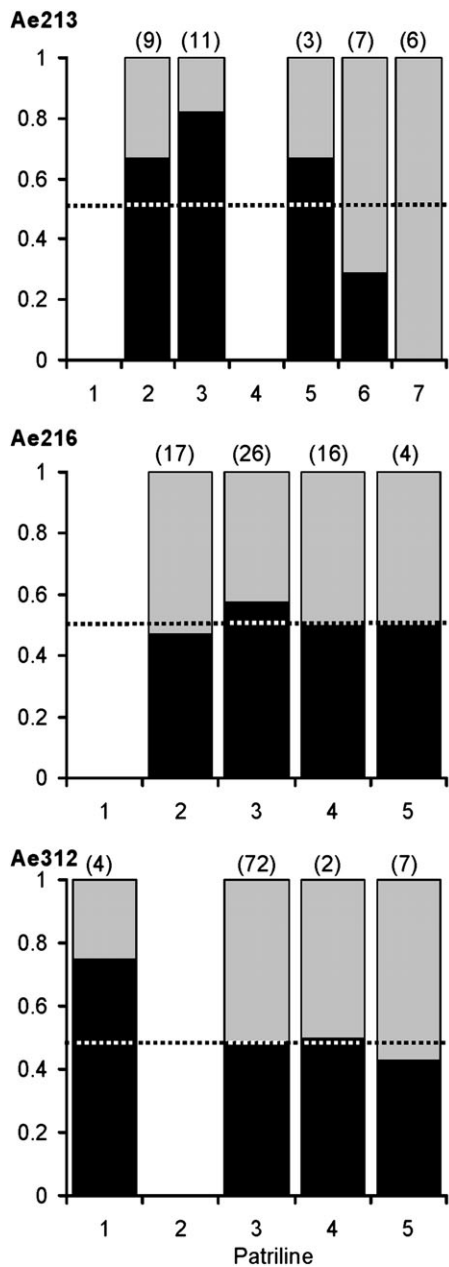
Figure 3
Proportions of individuals sampled per patriline from colonies Ae213, Ae216, and Ae312 that were original waste management workers (black) or which responded to engage in waste management following an experimental increase in stimulus (gray). The dashed lines show the expected ratio given the numbers of individuals genotyped if the responding individuals were from the same patrilines as the original waste management workers (lines differ slightly from 0.5 because not all individuals could be assigned to patrilines). Sample sizes are given in parentheses above the columns for each patriline. Rare patrilines with less than 3 workers have been excluded.

stimuli during the development of larvae into morphological workers castes (Hughes and Boomsma 2007).

There are 3 leading hypotheses for the evolution of polyandry in social insects: that it improves the disease resistance of colonies, dilutes genetically incompatible matings, and improves division of labor. Polyandry has previously been shown to make colonies of *A. echinatior* less susceptible to parasites

(Hughes and Boomsma 2004, 2006; Hughes et al. 2010) and will also benefit them by diluting genetically incompatible matings, such as those which result in diploid males (Dijkstra and Boomsma 2007). The results suggest that improved division of labor may be a further benefit of polyandry in *A. echinatior*. Previous work has shown there to be a genotypic influence on morphological caste and thus alloethism (Hughes et al. 2003; Hughes and Boomsma 2007), whereas the current results show there to also be a genotypic influence on behavior within castes. Workers in a colony belong to multiple patrilines that have different propensities to engage in particular tasks, and this may mean the colony responds more appropriately to changing task needs (Oldroyd and Fewell 2007; Oldroyd and Thompson 2007). Direct experimental evidence for this beneficial effect has so far only been obtained in honey bees (Jones et al. 2004; Mattila and Seeley 2007). However, our results show that the key prerequisite for the hypothesis, a genotypic influence on worker behavior, is present also in the leaf-cutting ant *A. echinatior*. It seems likely that genetic polyethism may be widespread in social insects and may have played a role in their multiple evolutions of polyandry.

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