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Size and asymmetry: are there costs to winning the royalty race?

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Abstract

Body size and morphology are key fitness-determining traits that can vary genotypically. They are likely to be important in social insect queens, which mate in swarms and found colonies independently, but genetic influences on queen morphology have been little investigated. Here, we show that the body size and morphology of queens are influenced by their genotype in the leaf-cutting ant *Acromyrmex echinator*, a species in which certain lineages (patrilines) bias their development towards reproductive queens rather than sterile workers. We found no relationship between the queen-worker skew of patrilines and the size or morphology of queens, but there was a significant relationship with fluctuating asymmetry, which was greater in more queen-biased patrilines. Our results suggest that queen-biased patrilines do not incur a fitness cost in terms of body size, but may face more subtle costs in developmental stability. Such costs may constrain the evolution of royal cheating in social insects.

Introduction

In plants and animals, body size and morphology are associated with a number of life-history traits, which include dispersal ability, survival probability, fecundity and mating success (Vander Meer *et al.*, 1992; Honěk, 1993; Carvalho *et al.*, 1998; Bonduriansky, 2001; Gonzaga & Vasconcellos-Neto, 2001; Jennions *et al.*, 2001; Araújo *et al.*, 2004; Kingsolver & Huey, 2008; Kovacs *et al.*, 2008; Kajita & Evans, 2010). Size is influenced by both genetic and environmental conditions, but in the insects it is the amount of food available to developing larvae that has the greatest effect on adult body size (Davidowitz *et al.*, 2003; Speight *et al.*, 2008). Many of the highly eusocial insect species are characterized by varied morphology, and individuals are often morphologically suited to a particular role. In some ant species, for example, females can develop into a variety of forms, ranging from small workers, generally employed within the nest, to soldiers, morphologically specialized to protect and defend the nest, and reproductive queens that may be hundreds of times larger than their smallest sisters (Hölldobler & Wilson, 1990). The variety of forms into which such genetically similar individuals are able to develop make this an important group in which to study body size and morphology.

Four main factors have been shown to affect brood development and hence body size in the social insects: genotype, maternal and sibsocial (worker) effects and environmental conditions (Wheeler, 1986; Mousseau & Fox, 1998; Hughes *et al.*, 2003; Schwander *et al.*, 2008; Radmacher & Strohm, 2010). Large queens often have a higher colony founding success and greater fecundity than small queens (Vander Meer *et al.*, 1992; Honěk, 1993; Wiernasz & Cole, 2003; Wenseleers *et al.*, 2005). In ants, body size is also associated with divergent dispersal and colony founding strategies. In general, large gynes (virgin queens) have high dispersal ability and high fat and glycogen reserves, a phenotype suited for independent colony foundation (Keller & Passera, 1988, 1989). Smaller gynes tend to mate and stay within the natal colony, join a ready established colony or found a colony close to the natal site with the aid of workers (Ross & Keller, 1995; Sündstrom, 1995; Rüppell *et al.*, 1998; Rüppell & Heinze, 1999; Peeters & Ito, 2001). It is likely that size dimorphism is a consequence of disruptive selection resulting from a trade-off between survival and

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reproductive output – independent colony foundation often has an extremely low success rate and puts individuals at high risk of predation (Schmid-Hempel, 1984; Hölldobler & Wilson, 1990), but queens that successfully found their own colony, on the whole, produce more offspring than those in polygynous nests (Vander Meer *et al.*, 1992).

A direct effect of genotype on body size has so far been demonstrated in only a few species: *Formica truncorum* (Bargum *et al.*, 2004), *Lasius niger* (Fjerdingstad, 2005), *Solenopsis invicta* (Ross & Keller, 1998) and *Pogonomyrmex badius* (Smith *et al.*, 2008). The complex social structure of ant societies means that it is often difficult to distinguish genetic effects from maternal and sibsocial effects (Linksvayer, 2006; Meunier & Chapuisat, 2009), but genetic effects can be demonstrated by looking at size variation in colonies headed by a single, multiply mated queen because individuals can be separated into a number of genetically distinct patrilines that differ only in their paternal genotype.

In an ant colony, larval food intake tends to be controlled by sterile female workers (Oster & Wilson, 1978) and so traits that are determined in the larval stage, such as body size, can be strongly affected by the social environment. In a colony with monomorphic sexuals, the body size of reproductive females can be the cause of intracolonial conflict of interest between the developing gynes and their nursing workers (Herbers, 1990; Backus, 1993) that is not dissimilar to the more traditional parent–offspring conflict (Trivers, 1972). Developing larvae can maximize their size, and hence direct fitness, by taking a disproportionate share of resources. In contrast, workers who, in general, can only facilitate the transmission of their genes through caring for sexual offspring produced by the colony (Bourke & Franks, 1995) will achieve greatest indirect fitness if all gynes develop to a standard optimum size, which may differ from a maximum potential size (Fjerdingstad, 2005). This logic would only be false if workers in polygynous or polyandrous colonies were able to preferentially rear the larvae to which they were most related. Nepotism has long been a controversial area of social insect research, and a conclusive demonstration of such behaviour in any social insect group is conspicuously absent (Queller *et al.*, 1990; DeHeer & Ross, 1997; Holzer *et al.*, 2006; Goodisman *et al.*, 2007; Zinck *et al.*, 2009). Currently, the best evidence for nepotism in a social insect comes from a study of *Formica fusca* in which the authors showed that in colonies with multiple queens, workers were able to favour their closest kin when rearing eggs and larvae (Hannonen & Sundstrom, 2003). Even this work, however, has suffered criticism as the findings can also be explained by the variation in the egg viability of different queens, a phenomenon shown in polygynous colonies of *Formica exsecta* (Holzer *et al.*, 2006). Consequently, despite the evidence for chemical cues specific enough to allow such kin discrimination be present (Nehring *et al.*, 2011), nepotism is thought not to occur in colonies with multiple related matrilines or patrilines and so seems unlikely to play a role in queen size determination (Queller *et al.*, 1990; DeHeer & Ross, 1997).

Here, we investigate whether there is a genetic influence on queen size and morphology in the polyandrous leaf-cutting ant species *Acromyrmex echinatior* (Bekkevold *et al.*, 1999; Sumner *et al.*, 2004). *Acromyrmex echinatior* queens found colonies after their nuptial flight, and it is possible that body size may play an important role in all aspects of this process, from acquiring mates to founding a new colony and defending it from predators. Genetic effects on both queen-worker caste determination and worker caste development have been shown in *A. echinatior*, making this species a good model in which to detect genetic effects on within-caste morphology (Hughes *et al.*, 2003; Hughes & Boomsma, 2007, 2008). In addition, this species is particularly interesting because certain 'royal cheat' patrilines show a greater propensity to develop into gynes, thus cheating their nest-mates out of a fair chance of reproduction (Hughes & Boomsma, 2008). Such patriline variation in caste propensity can be due to the genetic incompatibilities in some ant species (Schwander & Keller, 2008), but the within-colony rarity of royal patrilines in leaf-cutting ants means that it is more likely to result from genotypic variation in the ability to obtain, utilize or respond to key caste-determining environmental cues, and fitness costs selecting for a low frequency of cheats within colonies (Hughes & Boomsma, 2008). Larval nutrition is one such environmental cue, and it may be that *Acromyrmex* cheats require less food to trigger gyne development, in which case we hypothesize that gynes from royal patrilines will be significantly smaller than those from other patrilines in the colony. We also predict that royal patrilines will show signs of developmental instability, resulting from potential nutritional or worker-induced stress during development. We assess this by examining fluctuating asymmetry (FA), the difference in size between right and left sides in organisms with bilateral symmetry (Van Valen, 1962), which is commonly used as a measure of developmental stability, an individual's ability to buffer development under imperfect environmental conditions (Palmer & Strobeck, 1986).

Materials and methods

We sampled gynes and workers from six monogynous *A. echinatior* colonies. Samples were collected from two colonies in the field (Ae125 and Ae158) and in the laboratory from four colonies (Ae07P4, Ae48, Ae357 and Ae088) that had been collected in Gamboa, Panama, and maintained in the laboratory at 26 °C and 80% RH, on a diet of privet leaves and rice. We sampled 96 gynes, 96 large workers and 96 small workers from each of Ae07P4, Ae357 and Ae088 and 96 gynes and 96 workers from each of Ae48, Ae125 and Ae158. For each colony, gyne and worker samples were all collected on the same day and were of similar cuticular coloration, ensuring that they were from the same brood cohort and differed little in age, such that any caste skew could not be explained by temporal changes in sperm use. Samples were stored in 100% ethanol at −20 °C. Samples from Ae48, Ae125 and Ae158 had previously been used in Hughes & Boomsma (2008).

We extracted DNA from the legs of individual ants using 5% Chelex 100 (BioRad, Hemel Hempstead, UK) suspended in 10 μM Tris buffer; 5 μL proteinase K (5 $\mu L\ mL^{-1}$) was added to the samples, which were then incubated at 56 °C overnight, then boiled for 15 min. All samples were genotyped at five polymorphic microsatellite loci: Ech1390, Ech3385, Ech4126, Ech4225 and Atco15 (Ortius-Lechner *et al.*, 2000; Helmkampf *et al.*, 2008). All PCR cycles had an initial denaturing step of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, an annealing step of 45 s and 72 °C for 45 s, with a final elongation step of 72 °C for 15 min completing the amplification process (for PCR conditions, see Table S1).

PCR products were run in an ABI 3130*x*1 capillary sequencer. Allele sizes were scored by comparison with internal size markers, and multilocus offspring genotypes were used to determine the genotypes of the colony queens and their multiple mates. This allowed us to assign individual workers and gynes to particular patrilines within each colony. Individuals for which paternity could not be reliably determined were excluded from the analysis (~4.5%). We used *G* tests for heterogeneity and Fisher's exact tests to examine whether the patrilines differed from the expected ratio in a uniform direction (Sokal & Rohlf, 1995). Tests for queen-worker skew and worker skew were conducted separately. Corrections for multiple testing were made using QVALUE software (Storey, 2002). Queen-worker skew for each patriline was calculated on a scale from −1 to 1, by subtracting the expected proportion of queens assuming no skew from the observed proportion of queens and multiplying by two.

Six body size measurements were made for each gyne sampled: head width, the maximum width across the eyes (HW); forewing length, from the first vein intersection to the base of the wing (WL); forewing width, running parallel to the cross-vein 2r (Brown & Nutting, 1950) (WW); maximum thorax length (TL); maximum thorax width (TW); and leg length, the fibula, patella and tibia (LL). Body parts were scanned using an Epson Scan V300 Photo (Epson UK Ltd, Hemel Hempstead, UK) with a resolution of 9600 pixels, with wings being scanned under glass slides. Measurements were made using IMAGEJ 1.42q (Rasband 1997–2011) and were calibrated using a scanned 0.1-mm graticule. To quantify measurement error, a randomized subset of 10 individuals were measured three times. Measurement error was estimated as the average coefficient of variation (CV) for each character, using Haldane's correction for small sample size (Haldane, 1955; Lynch & Hayden, 1995). The average measurement error of the six characters was < 1%.

We carried out all analyses in PASW Statistics 18 (IBM, Armonk, NY, USA), and measurements were log-transformed prior to analysis. One-sample Kolmogorov–Smirnov tests were used to test for normality within colony for each character. The length distribution of all characters measured did not differ significantly from a normal distribution.

The empirical morphospace – the distribution of realized forms (McGhee, 1991; Stone, 1997) – occupied by each colony was described by a principal components analysis (PCA) of the covariance matrix. Principal component (PC) axes with eigenvalues of at least 0.7 were retained (Jolliffe, 1972), and individual PC scores were subjected to a nested analysis of variance (ANOVA) with patriline nested within colony. Effect sizes (Cohen's *f*) were calculated using G*POWER v3.1.2 (Faul *et al.*, 2007).

We determined allometric relationships between head width, a trait commonly used as a measure of body size in ants (Hölldobler & Wilson, 1990), and all other characters using the equation $y = bx^{\alpha}$, where x and y are the size of the two given traits (Huxley & Teissier, 1936). The log transformation of this produces the linear equation $\log(y) = \log(b) + \alpha \log(x)$. The allometric coefficient (α), the scaling relationship between two traits, is the slope calculated from log–log plots of the two traits. If both traits are measured in the same dimension, an allometric coefficient of 1 indicates an isometric relationship, whereby the relative size of the traits does not vary with absolute size. Here, we used an ordinary least-squared regression to determine the allometric coefficient for each character against head width. A *t*-test was used to determine whether each allometric coefficient differed significantly from 1, and corrections for multiple testing were made using QVALUE software (Storey, 2002).

Both right and left wings of all individuals were measured twice for the FA analysis. Repeat measurements were made on separate days, and all measurements were made blind to patriline. The mean difference between right and left sides was used as a measure of asymmetry. Both directional asymmetry (DA), where one side of a trait has a propensity to develop more than the other, and antisymmetry (AS), whereby one side of a character is larger than the other but there is no bias as to which side is larger (Van Valen, 1962), can inflate estimates of FA (Palmer & Strobeck, 1986; Palmer, 1994). They can typically be recognized by skewed and platykurtic asymmetry frequency distributions, respectively. Before analysing FA, we tested the data for the presence of DA and AS, as recommended by Palmer (1994). Measurement error was partitioned from asymmetry using a mixed-model ANOVA, with side and replicate as fixed factors and individual as a random factor (Palmer, 1994; Swaddle *et al.*, 1994). Two FA indices were calculated for each patriline: absolute right minus left (FA1 from Palmer, 1994) and Palmer's (1994) FA10. FA10 is the only FA index that allows measurement error to be partitioned from the FA estimate (Palmer, 1994). The relationship between patriline FA and queen-worker skew was examined using a mixed model, with colony as a random factor.

Results

Patriline representation

Four of the six colonies genotyped contained patrilines that were over-represented in the queen caste, or 'royal patrilines' as defined by Hughes & Boomsma (2008); Fig. S1 and Table S2). Colonies Ae357 and Ae48 were each found to have one royal–LW patriline. Ae125 and Ae158 had three and two royal–SW patrilines, respectively.

Body size and morphology

The first three PCs extracted in the PCA accounted for a total of 86.4% of variation in the data (Table 1). In morphological studies, PCA typically results in a first component on which all traits are positively loaded, thus representing size (Blackith & Reyment, 1971; Bookstein, 1989). Here, we took PC1, which accounted for 56.1% of the total variation, as a measure of general body size. LL had a high positive loading on PC2, which explained 16.8% of the variation. TW had a strong positive loading on PC3, whereas all other characters had negative loadings on this component, which accounted for 13.5% of the total variation (Table 1). Mean PC scores for each patriline can be found in the (Table S3).

All three PCs analysed showed a significant difference between colonies (PC1: $F_{5,388} = 123.2$, $P < 0.001$; PC2: $F_{5,388} = 214.3$, $P < 0.001$; PC3: $F_{5,388} = 29.8$, $P < 0.001$) and between patrilines nested within colony (PC1: $F_{26,388} = 4.005$, $P < 0.001$; PC2: $F_{26,388} = 2.026$, $P < 0.01$; PC3: $F_{26,388} = 2.785$, $P < 0.001$; Fig. 1). The effect size of colony was large for all three PCs (PC1: $f = 0.831$; PC2: $f = 0.888$; PC3: $f = 0.62$). For patriline, averaged over all colonies, the effect size was medium for PC1 ($f = 0.231$) and PC3 ($f = 0.350$) but small for PC2 ($f = 0.139$) (Cohen, 1977). No significant relationship was found between any of the PCs and queen-worker skew (PC1: $r^2 = 0.014$, $F_{1,31} = 0.456$, $P = 0.51$; PC2: $r^2 = 0.015$, $F_{1,31} = 0.49$, $P = 0.49$; PC3: $r^2 = 0.0$, $F_{1,31} = 0.002$, $P = 0.97$; Fig. 2).

Table 1 Principal component (PC) loadings for the six traits used in the analysis.

	Components		
	PC1	PC2	PC3
Eigenvalue	3.37	1.01	0.81
Variance explained (%)	56.1	16.8	13.5
HW	0.845	0.121	−0.024
TL	0.905	−0.014	−0.033
TW	0.462	−0.108	0.859
WL	0.839	−0.353	−0.149
WW	0.800	−0.367	−0.215
LL	0.525	0.842	−0.047

The six morphological traits measured were head width (HW), thorax length (TL), thorax width (TW), wing length (WL), wing width (WW) and leg length (LL).

Thorax length was the only character measured that scaled isometrically with HW ($\alpha = 1.03$, $t_{418} = 0.56$, $P = 0.57$; Fig. 3a). TW showed the highest level of negative allometry ($\alpha = 0.38$, $t_{418} = 13.23$, $P < 0.0001$, $Q < 0.0001$; Fig. 3b). WL and WW showed some negative allometry ($\alpha = 0.76$, $t_{418} = 5.22$, $P < 0.0001$, $Q < 0.0001$; WW: $\alpha = 0.73$, $t_{418} = 5.56$, $P < 0.0001$, $Q < 0.0001$; Fig. 3c,d, respectively). LL also showed significant negative allometry ($\alpha = 0.77$, $t_{418} = 3.50$, $P < 0.001$, $Q < 0.00l$; Fig. 3e), although one colony, Ae48, had a disproportionate effect on this relationship (Ae48 only: $\alpha = 0.31$, $t_{85} = 10.80$, $P < 0.0001$, $Q < 0.0001$; Ae48 excluded: $\alpha = 0.88$, $t_{331} = 2.46$, $P < 0.05$, $Q < 0.01$).

Fluctuating asymmetry

We found no evidence of significant directional asymmetry or antisymmetry. Mean signed asymmetry did not differ significantly from 0 ($t_{425} = -1.078$, $P = 0.28$), signed asymmetry showed no evidence of a skewed or bimodal distribution, and the distribution of asymmetry did not differ significantly from a normal distribution ($Z = 0.11$, $P = 0.21$). Thus, deviations from perfect symmetry can be interpreted as FA. There was no significant relationship between trait size and asymmetry ($\rho = 0.02$, $P = 0.72$). Estimated asymmetry was found to be significantly greater than measurement error for the whole sample ($F_{423,846} = 10.74$, $P < 0.0001$) and also when each patriline was tested individually ($P < 0.001$ in all cases). FA1 and FA10 showed a strong positive relationship ($\rho = 0.77$, $P < 0.0001$), suggesting both offer a robust estimate of FA. There was a significant relationship between queen-worker skew and both FA1 and FA10, with more queen-biased patrilines exhibiting greater FA (FA1: $F_{1,25} = 10.0$, $P < 0.01$; Fig. 4; FA10: $F_{1,25} = 4.98$, $P < 0.05$).

Discussion

Here, we found a genetic effect on the body size of *A. echinatior* queens. In many insect taxa, including ants, large female body size is associated with high fecundity and reproductive output (Vander Meer *et al.*, 1992; Honěk, 1993), and in the ants, large queens are more likely to survive the initial stages of colony foundation (Wiernasz & Cole, 2003). It is therefore likely that in *A. echinatior*, as well as *P. badius*, *L. niger* and *F. truncorum* in which similar effects have been shown (Bargum *et al.*,

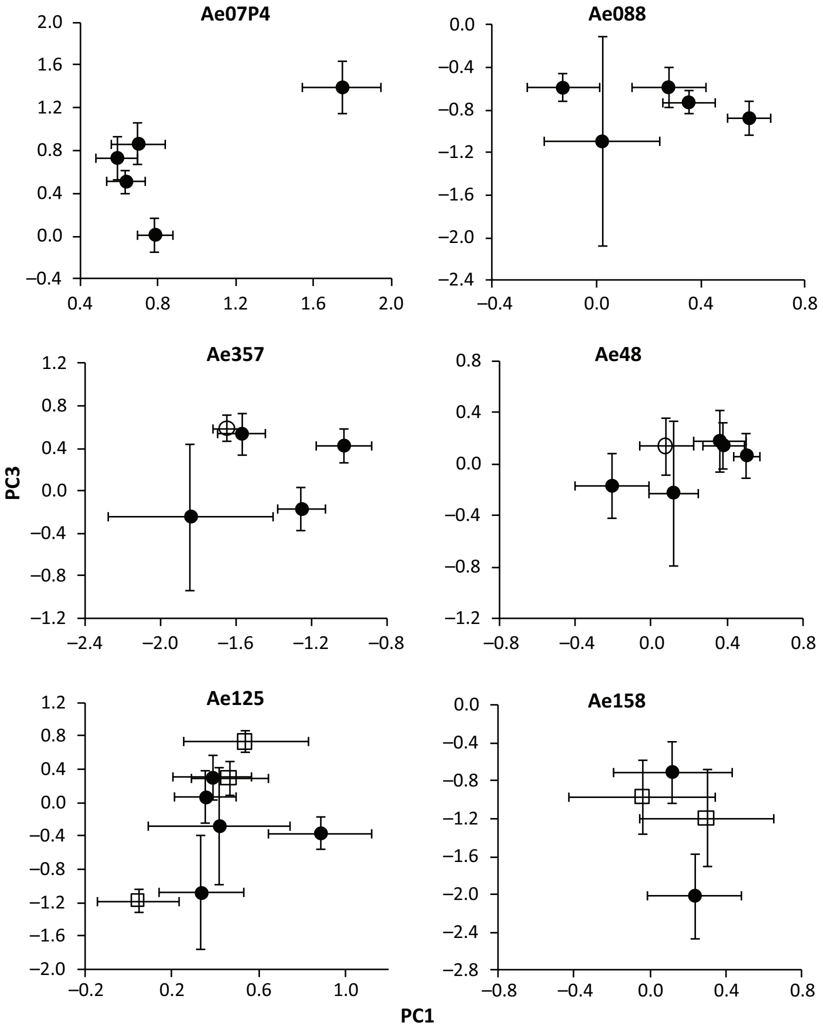

Fig. 1 Mean ± SEM PC1 and PC3 of patrilines from the six colonies used in the analysis. Black circles represent patrilines with no significant queen-worker bias. *Royal–LW* patrilines are represented by open circles, and *royal–SW* patrilines by open squares. PC, principal component.

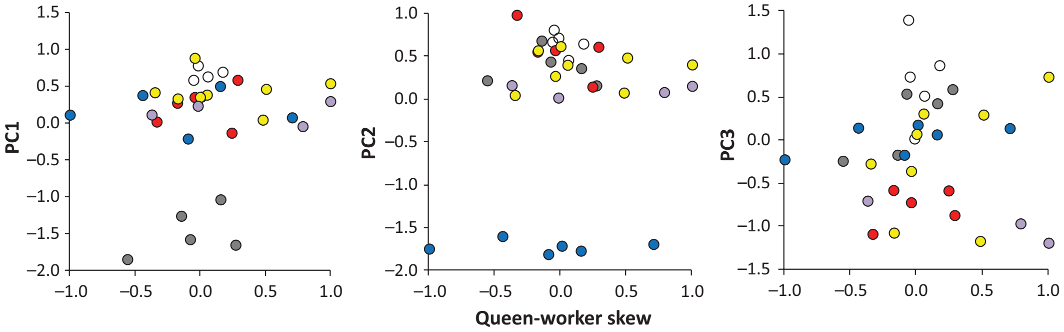

Fig. 2 Relationship between queen-worker skew and each of the first three principal components (PCs), each PC averaged by patriline. Each point represents one patriline, and each colour represents one of the six colonies. No significant relationship was found between queen-worker skew and any of the PCs analysed.

2004; Fjerdingstad, 2005; Smith *et al.*, 2008), large queens may have greater fitness than smaller individuals. Thus, those individuals who, as larvae, are better able to signal to workers that they require feeding, or those who are better able to use resources for growth, may be at a competitive advantage over their sisters.

The inclusive fitness of workers is strongly linked to colony productivity because the sexual offspring

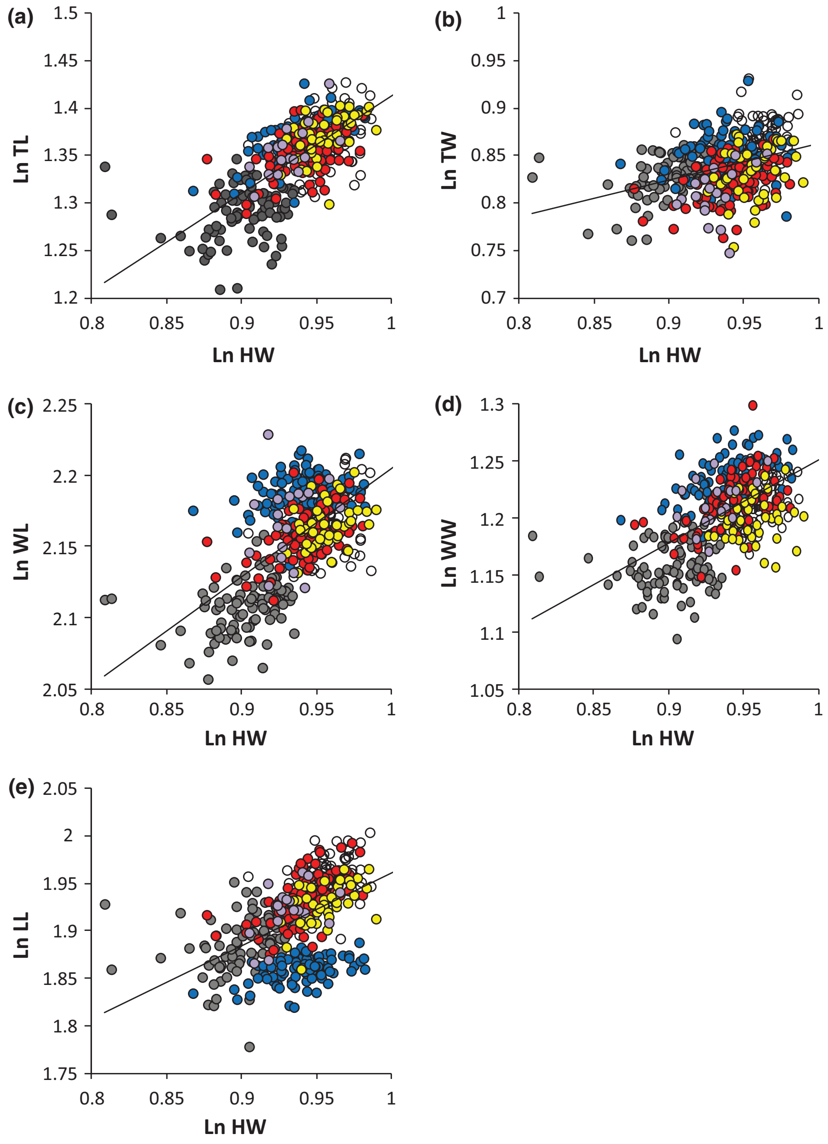

Fig. 3 Relationship between head width (HW) and five morphological traits, thorax length (TL), thorax width (TW), wing length (WL), wing width (WW) and leg length (LL). (a–e) Each circle represents one individual, and each colour corresponds to one of the six colonies. Lines of best fit are as follows: TL: $y = 1.03x + 0.39$; TW: $y = 0.38x + 0.49$; WL: $y = 0.76x + 1.44$; WW: $y = 0.73x + 0.52$; and LL: $y = 0.77x + 1.19$.

produced are generally the only means by which workers can contribute genes to the next generation (Hamilton, 1964). In the insects, adult body size is partly dependent on the amount of food received during development (Davidowitz *et al*., 2003; Speight *et al*., 2008), and in the social insects, it is the workers who forage and provide food for developing larvae. If a colony has limited resources with which to raise sexual offspring, workers, too, face a trade-off between the number and size of gynes produced. Thus, for workers, variation in the size of sexuals produced by the colony is not advantageous, unless workers possess the ability to recognize and preferentially rear their closest kin, something for which there is currently no conclusive evidence in social insects (Queller *et al*., 1990; DeHeer & Ross, 1997; Holzer *et al*., 2006; Goodisman *et al*., 2007; Zinck *et al*., 2009).

It has been proposed that royalty biasing of patrilines in *A. echinatior* could result from changes in response thresholds for environmental cues, such as nutrition, that control larval development (Hughes & Boomsma, 2008). Patrilines exhibiting a queen- and a large worker bias (*royal–LW* patriline) may have slightly lower nutritional thresholds for the initiation of both gyne and large worker development, whereas in patrilines with a queen- and small worker bias (*royal–SW* patrilines), it is only the queen-worker nutritional threshold that is reduced, but to a greater extent. Were this hypothesis correct, we might expect that, on average, gynes from these patrilines would have a smaller body size than those from normal patrilines, *royal–SW* patrilines differing more from the colony norm than *royal–LW* patrilines. The average body size of both the *royal–LW* patrilines was towards the

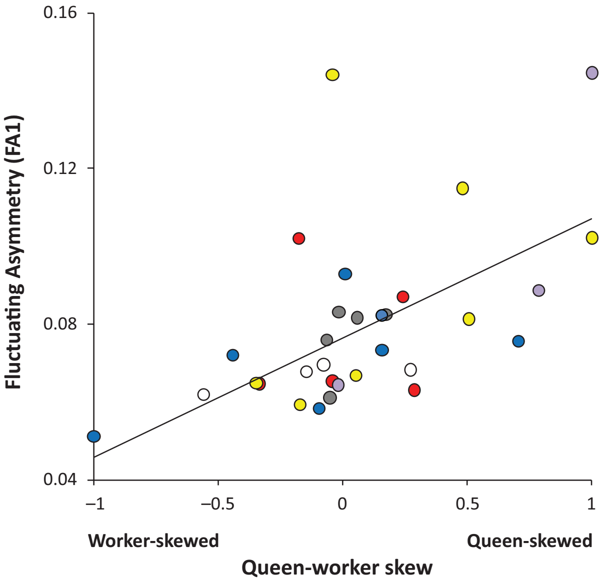

Fig. 4 Relationship between queen-worker skew and fluctuating asymmetry (FA1). Each point represents one patriline, and each colour represents one of the six colonies ($y = 0.031x + 0.076$, $r^2 = 0.319$, $F_{1,30} = 14.08$, $P < 0.001$).

low end of the size distribution (Fig. 1), but the lack of a similar relationship in *royal–SW* patrilines, in addition to a nonsignificant relationship between body size and queen-worker skew, means that the data presented here offer little support for this hypothesis. It may be therefore that the mechanism controlling caste determination is decoupled from that which determines queen size. In ant species that exhibit a worker polymorphism, such as *Acromyrmex*, the early divergence of queen and worker development may be crucial, allowing time for the development of worker size and allometric differences (Wheeler, 1986). In the fire ant *S. invicta*, gyne determination takes place in the first or second instar (Robeau & Vinson, 1976). In contrast, it is unlikely that adult body size is wholly determined at such an early stage of larval development and so the temporal separation of caste determination and morphological development may account for the lack of a relationship between size and queen-worker skew observed here.

In addition to information on body size, our results offer an interesting insight into morphological differences between *A. echinatior* queens. TL was the only character that scaled isometrically with head width. This is perhaps unsurprising, as both measurements are used as indicators of body size in insects (Hölldobler & Wilson, 1990; Berrigan, 1991; Partridge *et al.*, 1994). In contrast, WL, WW and LL showed some negative allometry. In holometabolous insects, adult appendages that develop from imaginal structures do not grow synchronously with the larval body, and so static allometries (allometry among body parts in a single species) in this group often differ from those expected based on other animal groups (Nijhout & Wheeler, 1996). The effect of colony on PC2, for which LL had a strong positive loading, was due to a single colony (Ae48) exhibiting a much greater negative allometry than the remainder of the sample. Interestingly, individuals from Ae48 had wings at the high end of the size distribution. The short legs and relatively large wings of queens in this colony could potentially result from appendages competing for nutrients during development (Nijhout & Wheeler, 1996). In winged insects, TW is a trait that may influence an individual's flight ability and consequently dispersal ability and reproductive success. Our results show a genetic effect on TW (PC3) and, surprisingly, TW showed negative allometry far higher than any other body part. This could result from a decoupling of the mechanism controlling the size of flight muscles and those involved in determining other components of body size, or from a restriction in the size of these muscles in terms of optimal flight ability.

Levels of FA have been investigated in a number of social insects, but previous studies have looked at variation in FA between castes, sex, colonies and species or subspecies (Ross & Robertson, 1990; Keller & Passera, 1993; Crespi & Vanderkist, 1997; Smith *et al.*, 1997; Heinze & Oberstadt, 1999; Fjerdingstad, 2004; Jones *et al.*, 2005). Here, we found a positive relationship between patriline queen-worker skew and FA. Such patriline variation in caste propensity in some ants can result from genetic incompatibilities (Schwander & Keller, 2008), but is more likely in *Acromyrmex* to be due to genotypic variation in the ability to obtain or utilize caste-determining environmental cues such as food (Hughes & Boomsma, 2008). FA is commonly used as a measure of developmental stability, an organism's ability to produce an ideal phenotype (perfect symmetry) under a particular set of environmental conditions (Zakharov, 1992). The relationship shown here could suggest that individuals from queen-biased patrilines have less ability to buffer environmental stress during development. This could result from a trade-off between ability to trigger gyne development and developmental stability. Alternatively, patriline variation in the amount of food required to trigger gyne development could lead to gynes from queen-biased patrilines being subject to greater levels of nutritional stress. Consequently, gynes from worker-skewed patrilines would be subject to the least nutritional stress, owing to a high nutritional threshold at which gyne development is triggered. Although our results suggest that there is no direct fitness cost from cheating in terms of reduced body size, it appears that there may be a more subtle cost to patriline caste biasing in *A. echinatior*, as indicated by the positive relationship between queen-worker skew and FA. It may be that cheats suffer costs in other fitness-related traits, such as offspring survival, immunity or learning ability (Jann &

Ward, 1999; Siva-Jothy & Thompson, 2002; Riddell & Mallon, 2006). Such costs may go some way towards balancing out the benefits gained by such individuals and may help to explain, in part, why cheats are rare. These results, however, should be interpreted with caution; although FA has been linked to a number of fitness traits, results are inconsistent and are often dependent on the trait in question (Lens & Van Dongen, 2002).

In conclusion, our results show a genetic effect on the size and morphology of queens in *A. echinatior*. In terms of body size, this is most likely to be a direct effect, resulting from between-patriline differences in the ability of larvae to signal to workers that they need food, or in their ability to process and utilize nutrients. However, an indirect genetic effect resulting from differential treatment by workers, although unlikely, cannot be ruled out. A strong colony effect on both body size and morphology suggests that although some variation in size is genetic, other influences, such as maternal effects, environmental variation and colony condition, are also important in determining these characteristics. Analysis of body size provided no direct evidence for the nutritional-threshold hypothesis, proposed by Hughes & Boomsma (2008) to explain the phenomenon of *A. echinatior* royal cheats, but a positive relationship between queen-worker skew and FA could be suggestive of individuals from queen-skewed patrilines suffering from greater nutritional stress as a result of a lower nutritional threshold for gyne development. It may be that experiments looking directly at preferential rearing by workers and how development is related to nutritional intake are the only way to conclusively test this hypothesis and determine the cause of caste bias in social insects.

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References

Araújo, E.D., Costa, M., Chaud-Netto, J. & Fowler, H.G. 2004. Body size and flight distance in stingless bees (Hymenoptera: Meliponini): inference of flight range and possible ecological implications. *Braz. J. Biol.* **64**: 563–568.

Backus, V.L. 1993. Packaging of offspring by nest of the ant *Leptothorax longispinosus*: parent offspring conflict and queen-worker conflict. *Oecologia* **95**: 283–289.

Bargum, K., Boomsma, J.J. & Sündstrom, L. 2004. A genetic component to size in queens of the ant, *Formica truncorum*. *Behav. Ecol. Sociobiol.* **57**: 9–16.

Bekkevold, D., Frydenberg, J. & Boomsma, J.J. 1999. Multiple mating and facultative polygyny in the Panamanian leafcutter ant *Acromyrmex echinatior*. *Behav. Ecol. Sociobiol.* **46**: 103–109.

Berrigan, D. 1991. The allometry of egg size and number in insects. *Oikos* **60**: 313–321.

Blackith, R.E. & Reyment, R.A. 1971. *Multivariate Morphometrics*. Academic Press, London.

Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.* **76**: 305–339.

Bookstein, F.L. 1989. ''Size and shape'': a comment on semantics. *Syst. Zool.* **38**: 173–180.

Bourke, A.F.G. & Franks, N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton: NJ.

Brown, W.L. Jr & Nutting, W.L. 1950. Wing venation and the phylogeny of the Formicidae (Hymenoptera). *Trans. Am. Entomol. Soc.* **75**: 113–132.

Carvalho, M.C., Queiroz, P.C.D. & Ruszczyk, A. 1998. Protandry and female size-fecundity variation in the tropical butterfly *Brassolis sophorae*. *Oecologia* **116**: 98–102.

Cohen, J. 1977. *Statistical Power Analysis for the Behavioural Sciences*. Academic Press, New York, NY.

Crespi, B.J. & Vanderkist, B.A. 1997. Fluctuating asymmetry in vestigial and functional traits of a haplodiploid insect. *Heredity* **79**: 624–630.

Davidowitz, G., D'Amico, L.J. & Nijhout, H.F. 2003. Critical weight in the development of insect body size. *Evol. Dev.* **5**: 188–197.

DeHeer, C.J. & Ross, K.G. 1997. Lack of detectable nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **40**: 27–33.

Faul, F., Erdfelder, E., Lang, A.G. & Buchner, A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **39**: 175–191.

Fjerdingstad, E.J. 2004. Multiple paternity and colony homeostasis in *Lasius niger* ants. *Behav. Ecol. Sociobiol.* **56**: 50–58.

Fjerdingstad, E.J. 2005. Control of body size of *Lasius niger* ant sexuals – worker interests, genes and environment. *Mol. Ecol.* **14**: 3123–3132.

Gonzaga, M.O. & Vasconcellos-Neto, J. 2001. Female body size, fecundity parameters and foundation of new colonies in *Anelosimus jabaquara* (Araneae, Theridiidae). *Insect. Soc.* **48**: 94–100.

Goodisman, M.A.D., Kovacs, J.L. & Hoffman, E.A. 2007. Lack of conflict during queen production in the social wasp *Vespula maculifrons*. *Mol. Ecol.* **16**: 2589–2595.

Haldane, J.B.S. 1955. The measurement of variation. *Evolution* **9**: 484.

Hamilton, W.D. 1964. The genetical evolution of social behaviour II. *J. Theor. Biol.* **7**: 17–52.

Hannonen, M. & Sundstrom, L. 2003. Worker nepotism among polygynous ants. *Nature* **421**: 910.

Heinze, J. & Oberstadt, B. 1999. Worker age, size and social status in queenless colonies of the ant *Leptothorax gredleri*. *Anim. Behav.* **58**: 751–759.

Helmkampf, M., Gadau, J. & Feldhaar, H. 2008. Population- and sociogenetic structure of the leaf-cutter ant *Atta colombica* (Formicidae, Myrmicinae). *Insect. Soc.* **55**: 434–442.

Herbers, J.M. 1990. Reproductive investment and allocation ratios for the ant *Leptothorax longispinosus*: sorting out the variation. *Am. Nat.* **136**: 178–208.

Hölldobler, B. & Wilson, E.O. 1990. *The Ants*. Belknap Press of Harvard University Press, Cambridge: MA.

Holzer, B., Kummerli, R., Keller, L. & Chapuisat, M. 2006. Sham nepotism as a result of intrinsic differences in brood viability in ants. *P. Roy. Soc. Lond. B. Biol. Sci.* **273**: 2049–2052.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**: 483–492.

Hughes, W.O.H. & Boomsma, J.J. 2007. Genetic polymorphism in leaf-cutting ants is phenotypically plastic. *P. Roy. Soc. Lond. B. Biol. Sci.* **274**: 1625–1630.

Hughes, W.O.H. & Boomsma, J.J. 2008. Genetic royal cheats in leaf-cutting ant societies. *Proc. Natl Acad. Sci. USA* **105**: 5150–5153.

Hughes, W.O.H., Sumner, S., Van Borm, S. & Boomsma, J.J. 2003. Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. *Proc. Natl Acad. Sci. USA* **100**: 9394–9397.

Huxley, J.S. & Teissier, G. 1936. Terminology of relative growth. *Nature* **137**: 780–781.

Jann, P. & Ward, P.I. 1999. Maternal effects and their consequences for offspring fitness in the Yellow Dung Fly. *Funct. Ecol.* **13**: 51–58.

Jennions, M.D., Moller, A.P. & Petrie, M. 2001. Sexually selected traits and adult survival: a meta-analysis. *Q. Rev. Biol.* **76**: 3–36.

Jolliffe, I.T. 1972. Discarding variables in a principal component analysis I: artificial data. *J. R. Stat. Soc. Ser. C Appl. Stat.* **21**: 160–173.

Jones, J.C., Helliwell, P., Beekman, M., Maleszka, R. & Oldroyd, B.P. 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. *J. Comp. Physiol. A.* **191**: 1121–1129.

Kajita, Y. & Evans, E.W. 2010. Relationships of body size, fecundity, and invasion success among predatory lady beetles (Coleoptera: Coccinellidae) inhabiting alfalfa fields. *Ann. Entomol. Soc. Am.* **103**: 750–756.

Keller, L. & Passera, L. 1988. Energy investment in gynes of the Argentine ant *Iridomyrmex humilis* (Mayr) in relation to the mode of colony founding in ants (Hymenoptera: Formicidae). *Int. J. Invertebr. Reprod. Dev.* **13**: 31–38.

Keller, L. & Passera, L. 1989. Size and fat content in gynes in relation to the mode of colony founding in ants (Hymenoptera, Formicidae). *Oecologia* **80**: 236–240.

Keller, L. & Passera, L. 1993. Incest avoidance, fluctuating asymmetry, and the consequences of inbreeding in *Iridomyrmex humilis*, an ant with multiple queen colonies. *Behav. Ecol. Sociobiol.* **33**: 191–199.

Kingsolver, J.G. & Huey, R.B. 2008. Size, temperature, and fitness: three rules. *Evol. Ecol. Res.* **10**: 251–268.

Kovacs, J.L., Hoffman, E.A. & Goodisman, M.A.D. 2008. Mating success in the polyandrous social wasp *Vespula maculifrons*. *Ethology* **114**: 340–350.

Lens, L. & Van Dongen, S. 2002. Fluctuating asymmetry as a bio-indicator in isolated populations of the Taita thrush: a Bayesian perspective. *J. Biogeogr.* **29**: 809–819.

Linksvayer, T.A. 2006. Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution* **60**: 2552–2561.

Lynch, J.M. & Hayden, T.J. 1995. Genetic influences on cranial form: variation among ranch and feral American mink *Mustela vison*. *Biol. J. Linn. Soc.* **55**: 293–307.

McGhee, G.R. 1991. Theoretical morphology: the concept and its applications. In: *Analytical Paleobiology: Short Courses in Paleontology 4* (N.L. Gilinsky & P.W. Signor, eds), pp. 87–102. The Paleontological Society, Knoxville, TN.

Meunier, J. & Chapuisat, M. 2009. The determinants of queen size in a socially polymorphic ant. *J. Evol. Biol.* **22**: 1906–1913.

Mousseau, T.A. & Fox, C.W. 1998. The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**: 403–407.

Nehring, V., Evison, S.E.F., Santorelli, L.A., d'Ettorre, P. & Hughes, W.O.H. 2011. Kin-informative recognition cues in ants. *P. Roy. Soc. Lond. B. Biol. Sci.* **278**: 1942–1948.

Nijhout, H.F. & Wheeler, D.E. 1996. Growth models of complex allometries in holometabolous insects. *Am. Nat.* **148**: 40–56.

Ortius-Lechner, D., Gertsch, P.J. & Boomsma, J.J. 2000. Variable microsatellite loci for the leaf cutter ant *Acromyrmex echinatior* and their applicability to related species. *Mol. Ecol.* **9**: 114–116.

Oster, G.F. & Wilson, E.O. 1978. *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton: NJ.

Palmer, A.R. 1994. Fluctuating asymmetry analyses: a primer. In: *Developmental Instability: Its Origins and Evolutionary Implications* (T.A. Markow, ed.), pp. 335–364. Kluwer, Dordrecht.

Palmer, A.R. & Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis and patterns. *Ann. Rev. Ecol. Syst.* **17**: 391–421.

Partridge, L., Barrie, B., Fowler, K. & French, V. 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**: 1269–1276.

Peeters, C. & Ito, F. 2001. Colony dispersal and the evolution of queen morphology in social hymenoptera. *Ann. Rev. Entomol.* **46**: 601–630.

Queller, D.C., Hughes, C.R. & Strassmann, J.E. 1990. Wasps fail to make distinctions. *Nature* **344**: 388.

Radmacher, S. & Strohm, E. 2010. Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie* **41**: 169–177.

Rasband, W.S. 1997–2011. *ImageJ*. US National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/.

Riddell, C.E. & Mallon, E.B. 2006. Insect psychoneuroimmunology: immune response reduces learning in protein starved bumblebees (*Bombus terrestris*). *Brain Behav. Immun.* **20**: 135–138.

Robeau, R. & Vinson, S.B. 1976. Effects of juvenile hormone analogues on caste differentiation in the imported fire ant, *Solenopsis invicta*. *J. Georgia Entomol. Soc.* **11**: 198–202.

Ross, K.G. & Keller, L. 1995. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. *Ann. Rev. Ecol. Syst.* **26**: 631–656.

Ross, K.G. & Keller, L. 1998. Genetic control of social organization in an ant. *Proc. Natl Acad. Sci. USA* **95**: 14232–14237.

Ross, K.G. & Robertson, J.L. 1990. Developmental stability, heterozygosity, and fitness in two introduced fire ants (*Solenopsis invicta* and *S. richteri*) and their hybrid. *Heredity* **64**: 93–103.

Rüppell, O. & Heinze, J. 1999. Alternative reproductive tactics in females: the case of size polymorphism in winged ant queens. *Insect. Soc.* **46**: 6–17.

Rüppell, O., Heinze, J. & Hölldobler, B. 1998. Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (Emery). *Insect. Soc.* **45**: 67–77.

Schmid-Hempel, P. 1984. Individually different foraging methods in the desert ant *Cataglyphis bicolor* (Hymenoptera, Formicidae). *Behav. Ecol. Sociobiol.* **14**: 263–271.

Schwander, T. & Keller, L. 2008. Genetic compatibility affects queen and worker caste determination. *Science* **322**: 552.

Schwander, T., Humbert, J.Y., Brent, C.S., Cahan, S.H., Chapuis, L., Renai, E. *et al.* 2008. Maternal effect on female caste determination in a social insect. *Curr. Biol.* **18**: 265–269.

Siva-Jothy, M.T. & Thompson, J.J.W. 2002. Short-term nutrient deprivation affects immune function. *Physiol. Entomol.* **27**: 206–212.

Smith, D.R., Crespi, B.J. & Bookstein, F.L. 1997. Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. *J. Evol. Biol.* **10**: 551–574.

Smith, C.R., Anderson, K.E., Tillberg, C.V., Gadau, J. & Suarez, A.V. 2008. Caste determination in a polymorphic social insect: nutritional, social, and genetic factors. *Am. Nat.* **172**: 497–507.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*. W H Freeman and Company, New York, NY.

Speight, M.R., Hunter, M. & Watt, A. 2008. *Ecology of Insects*. Blackwell, Oxford.

Stone, J.R. 1997. The spirit of D'Arcy Thompson dwells in empirical morphospace. *Math. Biosci.* **142**: 13–30.

Storey, J.D. 2002. A direct approach to false discovery rates. *J. R. Stat. Soc. B* **64**: 479–498.

Sumner, S., Hughes, W.O.H., Pedersen, J.S. & Boomsma, J.J. 2004. Ant parasite queens revert to mating singly. *Nature* **428**: 35–36.

Sündstrom, L. 1995. Dispersal polymorphism and physiological condition of males and females in the ant *Formica truncorum*. *Behav. Ecol.* **6**: 132–139.

Swaddle, J.P., Witter, M.S. & Cuthill, I.C. 1994. The analysis of fluctuating asymmetry. *Anim. Behav.* **48**: 986–989.

Trivers, R.L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (B. Campbell, ed.), pp. 136–179. Heineman, London.

Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* **16**: 125–142.

Vander Meer, R.K., Morel, L. & Lofgren, C.S. 1992. A comparison of queen oviposition rates from monogyne and polygyne fire ant, *Solenopsis invicta*, colonies. *Physiol. Entomol.* **17**: 384–390.

Wenseleers, T., Ratnieks, F.L.W., Ribeiro, M.D., Alves, D.D. & Imperatriz-Fonseca, V.L. 2005. Working-class royalty: bees beat the caste system. *Biol. Lett.* **1**: 125–128.

Wheeler, D.E. 1986. Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am. Nat.* **128**: 13–34.

Wiernasz, D.C. & Cole, B.J. 2003. Queen size mediates queen survival and colony fitness in harvester ants. *Evolution* **57**: 2179–2183.

Zakharov, V.M. 1992. Population phenogenetics: analysis of developmental stability in natural populations. *Acta Zool. Fenn.* **191**: 7–30.

Zinck, L., Chaline, N. & Jaisson, P. 2009. Absence of nepotism in worker-queen care in polygynous colonies of the ant *Ectatomma tuberculatum*. *J Insect Behav* **22**: 196–204.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Conditions for the amplification of five polymorphic microsatellites used to identify patrilines in each colony of *Acromyrmex echinatior*.

Table S2 Results of Fisher's exact tests (all patrilines) and G tests (only the more abundant patrilines) comparing actual frequencies of queens and workers with the frequencies expected for that colony.

Table S3 Mean (SEM) PC1, PC2 and PC3 of patrilines from each of the six colonies used in the analysis. *N* is the number of gynes sampled from each patriline.

Figure S1 The proportion of individuals sampled per patriline for six colonies that were workers (clear) or queens (coloured).

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