Qualitative bias in offspring investment in a superorganism is linked to dispersal and nest inheritance

Adam L. Cronin [a, *], Thibaud Monnin [b], David Sillam-Dussès [b, c, d], Fabien Aubrun [b], Pierre Fédérici [b], Claudie Doums [e, f]

[a] United Graduate School of Agricultural Sciences, Iwate University, Morioka, Japan
[b] Sorbonne Universités, UPMC Univ Paris 06, UMR 7618 Institute of Ecology and Environmental Sciences of Paris, Paris, France
[c] Sorbonne Universités, Institut de Recherche pour le Développement, U242 Institute of Ecology and Environmental Sciences of Paris, Bondy, France
[d] Laboratoire d'Éthologie Expérimentale et Comparée, Université Paris 13–Sorbonne Paris Cité, Villetaneuse, France
[e] Institut de Systématique, Évolution, Biodiversité (ISYEB), EPHE, CNRS UPMC Univ Paris 06, MNHN, Sorbonne Universités, Paris, France
[f] EPHE, PSL Research University, Paris, France

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How parents invest resources in offspring is a central aspect of life history. While investment strategies have been well studied in solitary organisms, comparatively little has been done on social species, including the many that reproduce by fission. Under colony fission, a parent colony divides resources (individuals) to form two or more offspring colonies. Because individuals differ in characteristics (e.g. size), there is opportunity for both quantitative and qualitative bias in their allocation. In this study we investigated the qualitative aspect of offspring investment during colony fission. Colonies of the ant *Cataglyphis cursor* fission into multiple offspring colonies as part of their lifecycle, and the distribution of workers is quantitatively biased. We found that investment is also qualitatively biased in terms of worker size and worker genetic characteristics (patrilines). This bias was mainly between the offspring colony that inherited the original nest and offspring colonies that dispersed to new nesting sites. In 74% of cases, dispersing colonies contained larger workers, and the distribution of genetic patrilines was biased in two of six cases in a manner that cannot be explained by the observed variation in worker size between patrilines. Fission also led to a reduction in diversity in offspring colonies compared to the parent colony, in terms of both worker size (70% of cases) and genetic diversity (40% of cases). These patterns are probably the result of differing dispersal probability between workers of different patrilines and of different size. This differential allocation may be adaptive because larger workers may be of disproportionate value to dispersing colonies, and their loss an acceptable cost to the colony inheriting the nest.
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How individuals allocate resources during reproduction is a central focus of life history theory. While there can be advantages to producing larger offspring (Krist, 2011; Marshall & Keough, 2008), parents with finite resources face a trade-off between offspring size and number (Smith & Fretwell, 1974), and the vast literature exploring the influence of varied quantitative investment on offspring characteristics indicates that the optimal investment strategy is context dependent (Bernardo, 1996; Burgess, Bode, & Marshall, 2013). Less attention has been paid to the importance of qualitative variation in investment, although some authors have suggested this may be of at least equal importance (Krist, 2011; Mousseau & Fox, 1998; Nager, Monaghan, & Houston, 2000). For example, differential deposition of hormones in avian eggs can influence offspring phenotype and behaviour (reviewed in Gil, 2008; Groothuis, Müller, von Engelhardt, Carere, & Eising, 2005), and indeed, the available evidence suggests that variation in the composition of invested resources may have diverse effects in a wide range of species (De Fraipont, Clobert, John, & Meylan, 2000; Gil, 2008; McGraw, Adkins-Regan, & Parker, 2005).

Theoretical and empirical studies of offspring investment have focused on solitary organisms (Stearns & Hoekstra, 2005), while the vast and often ecologically dominant social fauna has suffered a dearth of attention. Social insect colonies are superorganisms, and new colonies are created through one of two different modes that differ strongly in the manner in which resources are allocated. Under independent colony foundation (ICF) young queens depart

alone from the parent colony, disperse and mate on the wing, and start new colonies on their own. Under this strategy, the quantity and quality (e.g. size, energetic resources) of individual sexual offspring (dispersing queens and males) produced by the colony will be the main determinant of colony reproductive success. In contrast, under dependent colony foundation (DCF, also known as swarming, budding or colony fission), young queens start new colonies with the help of nestmate workers (reviewed in: Cronin, Molet, Doums, Monnin, & Peeters, 2013; Peeters & Molet, 2010). These queens leave the parent colony accompanied by numerous workers and are continuously helped and protected. Colony reproductive success in this case depends largely on the number and characteristics of workers (e.g. age, size and genetic lineage) and other resources (such as brood, food and building material) allocated to the new colonies. However, despite the abundance and ecological importance of species employing DCF (Cronin et al., 2013), and the growing body of theoretical (Bulmer, 1983; Cronin, Loeuille, & Monnin, 2016; Crozier & Pamilo, 1996; Rangel, Reeve, & Seeley, 2013) and empirical (Amor et al., 2011; Briese, 1983; Chéron, Cronin et al., 2011; Cronin, Fédérici, Doums, & Monnin, 2012; Fernández-Escudero, Seppä, & Pamilo, 2001; Gotwald, 1995; Lenoir, Quérard, Pondicq, & Berton, 1988; Seeley, 1996; van Veen & Sommeijer, 2000) literature on the quantitative aspects of reproductive allocation during DCF, studies on qualitative variation during DCF in social insects have been largely restricted to assessing the potential for nepotism, for which no convincing evidence has been found (Breed, 2014; Heinze, Elsishans, & Hölldobler, 1997; Rangel, Mattila, & Seeley, 2009; Solís, Hughes, Klingler, Strassmann, & Queller, 1998).

In addition to the number of workers, an important consideration under DCF is the characteristics of the workers allocated to offspring nests. Workers are not identical entities, but can differ in a variety of ways including genetic makeup, experience, age and morphology. These factors are not necessarily independent, as for example some degree of genetic determination of worker morphology has been demonstrated in several species of ants (Fraser, Kaufmann, Oldroyd, & Crozier, 2000; Hughes, Sumner, Van Borm, & Boomsma, 2003; Jaffe, Kronauer, Kraus, Boomsma, & Moritz, 2007). Trait variation among workers means that they are not of equivalent fitness value for a new colony, and the number of workers allocated may thus be an incomplete measure of reproductive investment. Furthermore, because social organisms are subject to selection at multiple levels (Gardner & Grafen, 2009; Hölldobler & Wilson, 2009), the optimal allocation strategy from a colony perspective may not be the same as that from an individual perspective. A range of benefits to having a genetically diverse workforce has been demonstrated in social insects (Baer & Schmid-Hempel, 1999; Hughes & Boomsma, 2004; Seeley & Tarpy, 2007; Ugelvig, Kronauer, Schrempf, Heinze, & Cremer, 2010), and we might thus expect allocation to favour the maintenance of genetic diversity in newly produced colonies. However, despite the lack of evidence for nepotism, individual selection could potentially favour the association of workers with more closely related full-sister gynes (young queens), leading to reduced genetic diversity and thus the opposite pattern to that expected by colony level selection.

In this study, we investigated the potential for qualitative bias in reproductive allocation during DCF in a superorganism, using the thermophilic ant *Cataglyphis cursor*. This species reproduces exclusively via colony fission (a term we use here synonymously with DCF, following Cronin et al., 2013), with a parent colony producing on average four (range 2–7) offspring colonies containing a highly variable number of workers (Chéron, Cronin et al., 2011; Cronin et al., 2012; Lenoir et al., 1988). Workers exhibit continuous variation in size (Cagniant, 1983; Clémencet & Doums, 2007), an important trait in thermophilic ants because it is linked with

heat resistance and thus affects foraging behaviour and interspecific scramble competition (Cerdá, 2001; Cerdá & Retana, 1997). Colonies are headed by a single queen, mated with on average 5.6 ± 1.3 males (range 4–8; Pearcy, Aron, Doums, & Keller, 2004), and thus contain multiple worker patrilines (Chéron, Monnin, Fédérici, & Doums, 2011; Pearcy et al., 2004). In addition, although queens produce workers sexually and males by arrhenotokous parthenogenesis as in most Hymenoptera, this species is unusual in that new gynes (i.e. young virgin queens) can be produced either sexually or via thelytokous parthenogenesis (Doums et al., 2013; Pearcy et al., 2004).

Our investigation focuses on worker size and genetic patriline. From both of these perspectives, there are reasons to expect the pattern of allocation will deviate from our null hypothesis that workers are randomly distributed. First, worker size in ants is positively associated with higher longevity (Porter & Tschinkel, 1985), resilience to starvation (Heinze, Foitzik, Fischer, Wanke, & Kipyatkov, 2003), foraging efficiency (e.g. temperature resistance, Cerdá & Retana, 1997; Cerdá & Retana, 2000; Clémencet, Cournault, Odent, & Doums, 2010) and colony defence (Hölldobler & Wilson, 1990). However, as size increases so does production cost, and only well-established colonies may be able to produce the large workers they need. Newly founded colonies may thus benefit disproportionately from the presence of larger workers compared to larger, established colonies. We might therefore expect larger workers to be preferentially allocated to small colonies and/or colonies that disperse to new nesting sites (where a new nest must be excavated), with their corresponding depletion in the parent colony. Second, from a genetic standpoint, bias could arise if workers exhibit nepotistic tendencies and preferentially associate with gynes of their own patriline during fission for inclusive fitness purposes. However, in *C. cursor*, this can only occur in colonies with sexually produced gynes, because parthenogenetically produced gynes are equally related to all workers regardless of patriline. If nepotism does occur, we might therefore expect differences in patriline distribution of workers between sister colonies (i.e. offspring colonies derived from the fission of the same parent colony) containing sexually produced gynes from different patrilines. Alternatively, workers of different patrilines in bees and ants may exhibit different dispersal propensities (Kryger & Moritz, 1997; Seppä, Fernández-Escudero, Gyllenstrand, & Pamilo, 2008), and this could lead to bias in the distribution of patrilines between the offspring colony that inherited the nest and those that dispersed to new nesting sites. Finally, size-based allocation of workers may be confounded by genetic effects if these factors are related. Although genetic variation has not been convincingly linked to differences in worker size in two previous studies in *C. cursor* (Eyer, Freyer, & Aron, 2013; Fournier, Battaille, Timmermans, & Aron, 2008) this pattern has been demonstrated in other ant species (Evison & Hughes, 2011; Fraser et al., 2000; Hughes et al., 2003; Jaffe et al., 2007; Rheindt, Strehl, & Gadau, 2005) and we reassessed this possibility here.

METHODS

We used 54 offspring colonies of *C. cursor* from a previous study (Chéron, Cronin et al., 2011). These colonies were collected as products of the fission of 14 parent colonies in the field at Argelès-sur-Mer in southern France. 'Parent' and 'offspring' colonies refer to colonies before and after fission, respectively. That is, each parent colony divided into several offspring colonies, and offspring colonies derived from the same parent colony are thus 'sister' colonies. In *C. cursor* the queen of the monogynous parent colony (i.e. the mother queen) is retained in approximately half of all reproducing colonies (Chéron, Cronin et al., 2011) so that one of the resulting

offspring colonies may contain the original mother queen (who may also relocate from the original nest). Also note that because parent colonies cannot be sampled nondestructively we reconstructed them a posteriori based on their own offspring colonies (see below). We focused our analyses on two comparisons: (1) between offspring colonies and reconstructed parent colonies and (2) between offspring colonies, with particular focus on differences between dispersing colonies and those that continued to occupy the original nest.

Morphological Analysis

The morphometric data set comprised 1952 individuals from 54 offspring colonies, with 30–65 randomly sampled workers in each (excluding colony 544E, which contained only three workers; Table 1). The characteristics of each colony are given in Table 1 (see also Chéron, Cronin et al., 2011). Note that the genetic analysis (see below) indicated that the queen of colony 473B was not the mother queen as reported in Chéron, Cronin et al. (2011), but a gyne belonging to one of the patrilines found in workers. Colony 473B was therefore queenless.

As a measure of worker size, we used the length of the tibia of the right hindleg, as previous biometric studies on *C. cursor* indicated that all classical measures of worker size are highly intercorrelated ($r = 0.76$–0.92; Cagniant, 1983; Clémencet & Doums, 2007), and tibia length is correlated with worker resistance to temperature (Clémencet et al., 2010). Measurements were performed with NI Vision Assistant 7.0 (National Instruments Corp., Austin, TX, U.S.A.) using photographs taken with a Canon Powershot S80 camera mounted on a Leica MZ6 stereomicroscope.

Genetic Analysis

For the genetic analysis, we used a subset of 20 offspring colonies produced by the fission of six parent colonies (Table 1) and comprising a total of 745 workers and 20 gynes/queens. Four of the parent colonies produced only parthenogenetic gynes (426, 471, 511 and 634) and two produced at least one sexual gyne (present in offspring colonies 447C and 473B-E). For each of the 20 offspring colonies, workers used in morphological analyses were genotyped at six microsatellite loci (Ccur 11, Ccur 46, Ccur 58, Ccur 65, Ccur 99 and Ccur 100) to identify worker patrilines, following Chéron, Monnin et al. (2011). The genotypes of queens and gynes were obtained from the data set of Chéron, Monnin et al. (2011). However, whereas Chéron, Cronin et al. (2011) listed the gynes in offspring colonies 511A and 511D as sexually produced, reanalysis of these data indicates that these gynes were in fact parthenogenetically produced. In half of the cases (471, 511, 463), one offspring colony continued to occupy the original nest, whereas in the other cases (426, 447, 473) all colonies dispersed (Table 1).

To get a more precise estimate of the population allelic frequencies used to estimate relatedness, we added to the genetic data set one worker from 17 additional colonies collected the same year at the same site but which did not fission (Chéron, Cronin et al., 2011). Data were analysed using Relatedness 5.0.1 (Queller & Goodnight, 1989) weighting colonies equally.

Worker patrilines, and father genotypes, were determined for each colony using Matesoft (Moilanen, Sundström, & Pedersen, 2004). The probability of nondetection of a patriline because two males harboured the same allele combination by chance was low (0.0003; estimated following Boomsma & Ratnieks, 1996) as our loci are highly heterozygous (mean $He = 0.75$; Clémencet & Doums, 2007). For parent colonies in which the mother queen was missing (426, 447, 473, 634), her genotype was deduced from the parthenogenetically produced gynes and/or from worker genotypes using the 'broad' option in Matesoft. In all cases, a single mother queen genotype was possible (but see Results regarding a possible queen turnover in the parent colony 511).

Statistical Analysis

We first examined how the characteristics of workers in offspring colonies compared to the parent colony. For this purpose, it was necessary to reconstruct parent colonies because they could not be nondestructively sampled prior to fission and, as we only measured a subset of all available workers in offspring colonies, simply compiling data would not be representative of the parent colony as offspring colonies were of different size. We thus reconstructed a distribution of 1000 possible parent colonies by sampling from the measured and genotyped worker subsets as follows: all workers from the largest offspring colony were assigned to the reconstructed parent colony: For each remaining offspring colony,

Table 1
Colony metrics and collection data

Parent colony	Type of analyses	Offspring colonies								Total
		A	B	C	D	E	F	G	H	
426	M/G	–	62 (35)	153 (35-1F)	79 (35)	–	–	–	–	294 (105)
447	M/G	–	148 (35-1F)	112 (35)[S]	30 (30-2F)	–	–	–	–	290 (100)
456	M	1150 (35)	152 (35)	326 (35)	–	–	–	–	–	1628 (105)
464	M	72 (35)	180 (35)	–	–	–	–	–	–	252 (70)
471	M/G	492 (30)[Q]	137 (30)	160 (30)	–	–	–	–	–	789 (90)
473	M/G	–	78 (35)[S]	56 (35-3F)[S]	42 (35)[S]	102 (35-1F)[S]	–	–	–	278 (140)
511	M/G	394 (65)	890 (65)[Q]	–	45 (45-2F)	–	–	–	–	1329 (175)
544	M	243 (30)[Q]	305 (48)	276 (48)	213 (48)	3 (0)	–	–	–	1037 (174)
571	M	81 (35)	152 (35)	–	112 (35)	90 (35)	60 (35)	–	–	495 (175)
581	M	85 (35)	158 (35)[Q]	–	48 (18)	–	–	–	–	291 (88)
598	M	234 (35)	177 (35)	92 (35)	56 (35)	69 (35)	90 (35)[Q]	–	62 (35)	780 (245)
634	M/G	293 (35)	69 (35)	77 (35)	138 (30)	–	–	–	–	577 (135)
641	M	257 (35)	58 (35)	–	252 (35)	121 (35)	–	103 (35)	–	791 (175)
667	M	49 (35)	1284 (35)[Q]	160 (35)	71 (35)	–	244 (35)	–	–	1808 (175)

Number of workers collected and analysed in 54 offspring colonies studied by Chéron, Cronin et al. (2011). Offspring colonies are named with the label of their parent colony followed by a letter from A to H, with colonies designated 'A' (e.g. 456A) having inherited the nest of the parent colony and others (B−H) having dispersed to new nests. There was thus no colony A when all offspring colonies dispersed. The number of workers analysed from each offspring colony is given between parentheses. M and G indicate, respectively, whether morphological and genetic analyses were carried out, with the same workers used for both analyses. Foreign workers are indicated by values subtracted from the total sampled worker population and denoted 'F' and were not included in analyses. Each offspring colony contained either the mother queen (indicated by the superscript Q) or a gyne. Cases in which offspring colonies contained sexually produced gynes are marked with a superscript S whereas unmarked nests contained parthenogenetically produced gynes.

we then sampled individuals randomly without replacement, up to a number defined by that colony's proportional contribution to the parental colony relative to that of the largest offspring colony. For instance, for a parental colony of 500 workers that fissioned into two colonies of 200 and 300 workers and had 35 workers measured from each, the 35 measured workers of the largest colony (which comprised 60% of the parental colony) would be included in the reconstructed colony while a proportional number (40%) would be randomly sampled from the remaining colony (i.e. 23 workers for a total sample of 58 workers). The composition of reconstructed parental colonies was thus determined by the relative size of each offspring colony even though our sample sizes for genetic and morphological study were more or less consistent across nests and limited by the size of the largest offspring colony.

We estimated the distribution of patrilines and worker size in the parent colonies by generating for each metric a set of 1000 reconstructed colonies. We then considered whether observed values from offspring colonies lay within the 95% confidence intervals of the distribution of their reconstructed parent colonies. We used the same procedure for estimating the distribution of relatedness in reconstructed parent colonies, but because of limitations to processing time for relatedness calculations, our distribution comprised estimates from only 100 reconstructed colonies. Mean values for each metric analysed were also determined for each reconstructed parent colony and compared to the mean of their offspring colonies using paired Wilcoxon signed-rank tests.

For our morphometric analysis, we compared both mean worker size and diversity of worker size between offspring colonies and their reconstructed parent colony. The diversity of worker size was estimated using the unbiased coefficient of variation (cv) for small sample sizes (Sokal & Rohlf, 1995):

$$\mathrm{cv} = \left(1 + \frac{1}{4n}\right)\left(\frac{\mathrm{sd} \times 100}{m}\right)$$

Where n is the sample size (number of workers measured), and m and sd are, respectively, the mean and standard deviation of the tibia length. For the genetic analyses, we compared (1) the number of patrilines, (2) the relatedness estimate and (3) the skew in patriline distribution using the Nonacs B index (Nonacs, 2003). This index ranges from -1 to 1, with more positive values indicating higher bias than expected by chance (a value of 0) and more negative values indicating a more even distribution than expected by chance.

We next focused on the distribution of worker characteristics among sister colonies, i.e. offspring colonies derived from the same parent colony. To explore the influence of inheritance of the nest of the parent colony and presence of the mother queen we performed mixed-model analyses using the nlme package (Pinheiro & Bates, 2000) in R version 3.2.1 (R Core Team, 2014) with worker size as the response variable, inheritance of the parent nest and presence/absence of the mother queen as fixed factors, and offspring colonies within parent colonies as nested random factors. The mother queen was found in the offspring colony that inherited the parent nest in only two of the 14 cases (colonies 471A and 544A, Table 1). We could thus test the two effects in the same model but could not test for an interaction between the two factors as there was no case where the mother queen survived but the parent nest was not inherited. We also account for differences in worker size variation by using the weight function to allow for heterogeneous variance between offspring colonies that inherited the parent nest and those that dispersed. We report results from log-likelihood ratio tests between the full model and the model without the factor of interest. For testing fixed effects, models were fitted with maximum

likelihood (Pinheiro & Bates, 2000). The heterogeneous variance component was only retained in the full model if significant.

To compare patriline distributions between offspring colonies, we performed a Fisher exact test with the expectation that no bias should be found if worker allocation is random. Comparisons between new offspring colonies from the same parent colony were corrected for repeated tests following Benjamini and Hochberg (1995). We assessed the potential for nepotism in the form of a worker preference for gynes of the same patriline in the five colonies in which gynes had been sexually produced (447C and 473B-E; Table 1). We compared the proportion of workers of the focal gynes' patriline across all sister colonies using chi-square tests.

To explore possible links between patriline and morphology, we combined data for morphometric and genetic samples where available ($N = 20$ offspring colonies from six parent colonies) and analysed these with a mixed-effects model, with worker size as the response variable, patriline nested within parent colony and parent colony as random factors, and the number of workers of each patriline as a fixed covariate. We considered patriline as a random factor in analyses as our aim was to estimate the part of variation explained by patriline over the six colonies (as used for estimating heritability in animal models; Wilson et al., 2010), rather than to test whether specific patrilines within given colonies differed in worker size. To do so we compared models fitted with a restricted maximum likelihood with and without the effect of patriline nested within colony using a log likelihood ratio test (Pinheiro & Bates, 2000). The percentage of worker size variation explained by patriline was calculated from the random parameters of the mixed-effect model, and fixed effects were tested as previously described.

Finally, in the cases for which worker patriline distribution differed between sister colonies, the effects of worker size and patriline could be intertwined if patrilines that are more prone to leave the nests are also those with large workers. To test these factors simultaneously, we used the three parent colonies in which the parent nest was inherited by an offspring colony and tested whether patriline and worker size affected the propensity of workers to stay in the parent nest or leave to join a dispersing colony using a binomial glm (binomial function and logit link), with staying or leaving as the response variable. In this case, patriline was considered as a fixed factor, as we performed a separate analysis for each colony and there were too few patrilines to properly estimate the variance component. Factors were tested by comparing models with and without the factor of interest using chi-square tests (Crawley, 2007).

Ethical Note

Ants were observed without direct interference during the monitoring part of our study (see Chéron, Cronin et al., 2011). All specimens collected for morphological and genetic analysis were placed directly into 90% alcohol with 10% Tris-EDTA in accordance with standard sampling procedures.

RESULTS

Influence of Fission on Worker Size Distribution

We first compared worker size and coefficient of variation (cv) between offspring colonies and their reconstructed parent colony. The mean size of workers in offspring colonies differed from that expected if workers had been randomly allocated in 74% (40 of 54) of cases (i.e. was outside the 95% confidence intervals (CIs) of the reconstructed parent colonies; Fig. 1a). There was no significant difference in worker size between offspring colonies and the mean value of reconstructed parent colonies overall (Wilcoxon paired
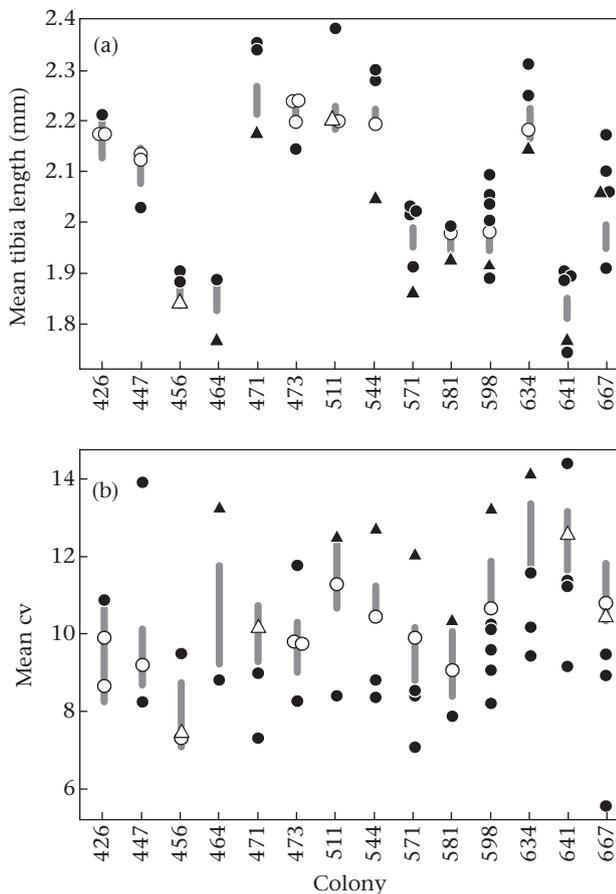
Figure 1. (a) Mean worker tibia length and (b) coefficient of variation in worker tibia length in offspring colonies that inherited the parent nest (triangles) and offspring colonies that dispersed (circles) compared to reconstructed parent colonies (dark-grey bars). Bars represent 95% CIs of 1000 reconstructed colonies. Black markers lie outside 95% CIs whereas white markers lie within 95% CIs. Note that in some cases, all colonies dispersed and no colony inherited the parent nest.

test: $z = -1.54$, $N = 14$, $P = 0.124$). None the less, workers in offspring colonies that inherited the parent nest were on average smaller than in the reconstructed parent colony ($z = -2.22$, $N = 11$, $P = 0.026$) whereas workers in offspring colonies that dispersed to new nesting sites tended to be larger than those in the reconstructed parent colony ($z = -1.96$, $N = 14$, $P = 0.050$; Fig. 1a). Similarly, the cv in worker size differed from the distribution of reconstructed parent colonies in 70% (38 of 54) of offspring colonies (Fig. 1b). While the pattern among offspring colonies was again nonsignificant (Wilcoxon paired test: $z = -1.66$, $N = 14$, $P = 0.096$), offspring colonies that inherited the parent nest tended to have a higher cv ($z = -1.78$, $N = 11$, $P = 0.075$) while those that dispersed had a lower cv than that of the reconstructed parent colony ($z = -2.23$, $N = 14$, $P = 0.026$).

We then focused on differences between sister colonies. There was no heterogeneity of variance in worker size linked to the presence of the mother queen (likelihood ratio $= 0.038$, $df = 1$, $P = 0.85$) whereas there was significant heterogeneity of variance linked to the inheritance of the parent nest (likelihood ratio $= 17.57$, $df = 1$, $P < 0.0001$), with variance being 1.4 times higher in offspring colonies that dispersed than in those that inherited the parent nest (Fig. 1b). The presence of the mother queen had no effect on mean worker size (likelihood ratio $= 1.88$, $df = 1$, $P = 0.17$). However, workers in the offspring colony that inherited the parent nest were smaller on average than those in offspring colonies that dispersed (likelihood ratio $= 16.15$, $df = 1$, $P < 0.0001$; Fig. 1a).

Influence of Fission on Genetic Characteristics

Our genetic analysis indicated that six offspring colonies contained a total of 10 foreign workers (Table 1). Following removal of these workers from the genetic data set, we obtained a total of 735 genotyped workers from 20 offspring colonies produced by six parent colonies. We identified between three and 12 patrilines per parent colony. Two of these patrilines contained a single individual each, but in both cases the putative father differed by at least two loci from the other putative fathers of the colony so that they were considered real patrilines and not results of PCR error.

The number of patrilines in reconstructed parent colonies (6.7 ± 3.3, range 3–12; based on median values of reconstructed colonies) was in most cases no different to that measured in offspring colonies (mean $= 6.2 \pm 2.5$, range 3–9; Fig. 2a) and there was no difference in mean values between parent and offspring colonies overall (Wilcoxon paired test: $z = -1.60$, $N = 6$, $P = 0.109$). The frequency of workers of different patrilines was highly skewed in the 20 offspring colonies, as well as in their reconstructed parent colonies (Nonacs skew index $B > 0$; $P < 0.001$ in all cases; Fig. 2b). Skew in 40% (eight of 20) of offspring colonies differed significantly from that of their reconstructed parent colony (Fig. 2b), and was higher in six of these cases, representing a significant pattern of increased skew overall following colony fission (Wilcoxon paired test: $z = -2.20$, $N = 6$, $P = 0.028$). Relatedness in reconstructed parent colonies ranged from 0.279 to 0.575 (median values; Fig. 2c) and in offspring colonies from 0.268 to 0.592. Relatedness in the latter differed from the distribution of the 100 reconstructed parent colonies in 40% (eight of 20) of cases, and was in all instances higher than expected if workers were randomly allocated, representing a pattern of increased relatedness following fission overall (Wilcoxon paired test: $z = -2.20$, $N = 6$, $P = 0.028$; Fig. 2c). In contrast to our results for morphology there was no clear pattern of differences between colonies that inherited the original nest and those that dispersed, although differences between parent and offspring colonies were more common in cases in which one colony continued to occupy the original nest (30%, 70% and 60% for patrilines, relatedness and skew respectively; $N = 10$) than those in which all colonies dispersed (0%, 10% and 20% respectively; $N = 10$).

The distribution of worker patrilines differed significantly (i.e. was not random) between sister colonies in only two of the six cases (Fig. 3). For these two cases, pairwise tests revealed significant differences between the colonies that inherited the parent nest and three dispersing colonies (following adjustments for multiple tests; Benjamini & Hochberg, 1995; Fig. 3) as well as between dispersing colonies 511B and 511D. In the latter case the difference resulted from the absence of four patrilines in colony 511B relative to 511D, and a close look at the genetic data strongly suggests the occurrence of a queen turnover in the parent colony in the previous season (as the current mother queen had already produced workers) or the fusion of two colonies headed by two clonal queens. Indeed, for four patrilines, only one of the two alleles of the current mother queen was found in the workers, suggesting the mother of these patrilines was homozygous at these loci, unlike the current mother queen. Interestingly, these four patrilines were absent from colony 511B where the current mother queen was found.

Evidence for Nepotism

We investigated the propensity for workers to associate with a gyne of their own patriline in the five offspring colonies with sexually produced gynes (447C, 473B-E; Table 1). In each case the proportion of workers of the same patriline as the gyne was no different in the colony where the gyne was located than in sister
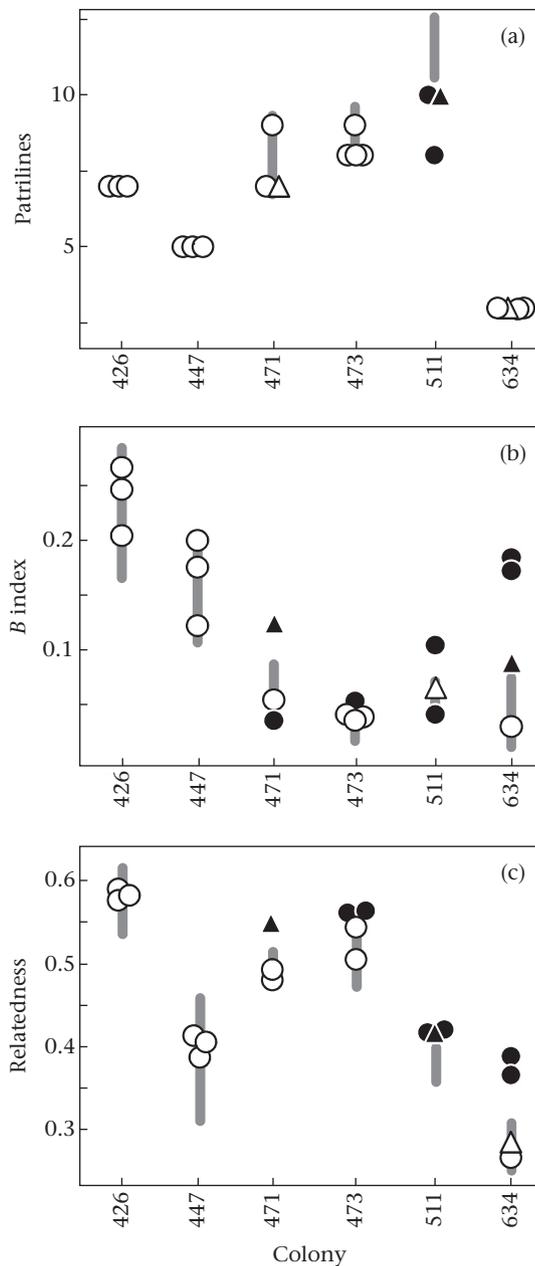
Figure 2. (a) Number of patrilines, (b) mean skew (B index) in patriline distribution and (c) relatedness values for offspring colonies that inherited the parent nest (triangles) and offspring colonies that dispersed (circles) compared to distributions for reconstructed parent colonies (dark-grey bars). Bars represent 95% CIs of 1000 reconstructed colonies (or 100 reconstructed colonies for relatedness). Black markers lie outside 95% CIs whereas white markers lie within 95% CIs. Note that in some cases, all colonies dispersed and no colony inherited the parent nest.

colonies (chi-square tests: $P > 0.28$ in all cases), suggesting workers are not disproportionately associated with gynes of the same patriline during fission.

Effect of Patriline on Worker Size

In the 20 offspring colonies for which we had both genetic and morphological data, the number of workers of each patriline was not associated with worker size (likelihood ratio $= 2.28$, $df = 1$, $P = 0.13$) and this factor was thus removed from the model. Workers of different patrilines varied in size (likelihood

ratio $= 21.13$, $df = 1$, $P < 0.001$), with patriline explaining 7.7% of the variation in worker size. None the less, in the cases for which we detected differences in worker patriline distribution between sister colonies, the effects of worker size and patriline could be intertwined if patrilines that are more prone to leave the nests are also those with large workers. For the three cases in which an offspring colony inherited the nest, we tested whether patriline and worker size affected the propensity of workers to stay in the inheriting colony or leave with a dispersing colony using a binomial model with staying or leaving as the response variable. In the two cases (colonies 511 and 634) with a significant difference in patriline distribution, worker propensity to leave was significantly associated with both patriline ($\chi^2_8 = 12.35$, $P < 0.0001$ for colony 511 and $\chi^2_2 = 20.58$, $P < 0.0001$ for colony 634) and worker size ($\chi^2_1 = 11.99$, $P < 0.001$ for colony 511 and $\chi^2_1 = 7.38$, $P = 0.007$ for colony 634). On the other hand, in colony 471 for which we previously did not detect significant variation in patriline distribution between sister colonies, there was no effect of patriline ($\chi^2_8 = 12.35$, $P = 0.14$) but a highly significant effect of worker size on the propensity to leave ($\chi^2_1 = 12.95$, $P < 0.001$). These results indicate that both patriline and worker size can independently influence the propensity to leave the parent nest.

DISCUSSION

Workers were not allocated randomly during the process of colony fission in the ant *C. cursor*, but according to worker size, and in some cases patriline. Specifically, larger workers were more likely to be allocated to offspring colonies that dispersed than remain in the offspring colony that inherited the parent nest. We also showed by comparing offspring colonies and reconstructed parent colonies that in many cases fission led to a reduction in the diversity of worker size and genetic diversity in offspring colonies. Finally, workers of different patrilines differed in size, in contrast to two previous studies on this species (Eyer et al., 2013; Fournier et al., 2008) but in support of studies on other ants (Fraser et al., 2000; Hughes et al., 2003; Jaffe et al., 2007). Our findings therefore indicate that allocation of workers is not only quantitatively biased (Chéron, Cronin et al., 2011), but also qualitatively biased, and this is linked with nest inheritance and dispersal. These findings align with data on fission in honeybees, *Apis mellifera*, that show that the departing swarm containing the mother queen has a quantitative advantage in incorporating the majority of the workforce, while a qualitative advantage extends to the nonswarming daughter colony in the form of the inherited material resources represented by the nest comb, its brood and provisions (Rangel & Seeley, 2012).

During colony fission in *C. cursor*, resources (adults and brood) are carried individually from the parent colony to offspring colonies by a proportion of workers acting as scouts (Cagniant, 1976; Lenoir et al., 1988). As in other social insects employing DCF, these scouts also act as decision makers in determining to which offspring colony resources will be allocated (Seeley & Morse, 1977). Thus, whereas the passive majority of individuals may have some control over whether they stay in the colony that inherits the parent nest or depart to join a dispersing colony (for example by placing themselves at the nest entrance), they have little choice over the destination. Bias between the offspring colony inheriting the nest and dispersing colonies can thus arise in two ways: (1) differential dispersal propensities between workers, with some ants actively (or incidentally) adopting behaviours that increase their chances of being carried to a dispersing colony and (2) active discrimination by transporting scouts, in selecting workers to be carried to dispersing colonies on the basis of particular characteristics. These modes of generating bias may act in concert or independently, although we
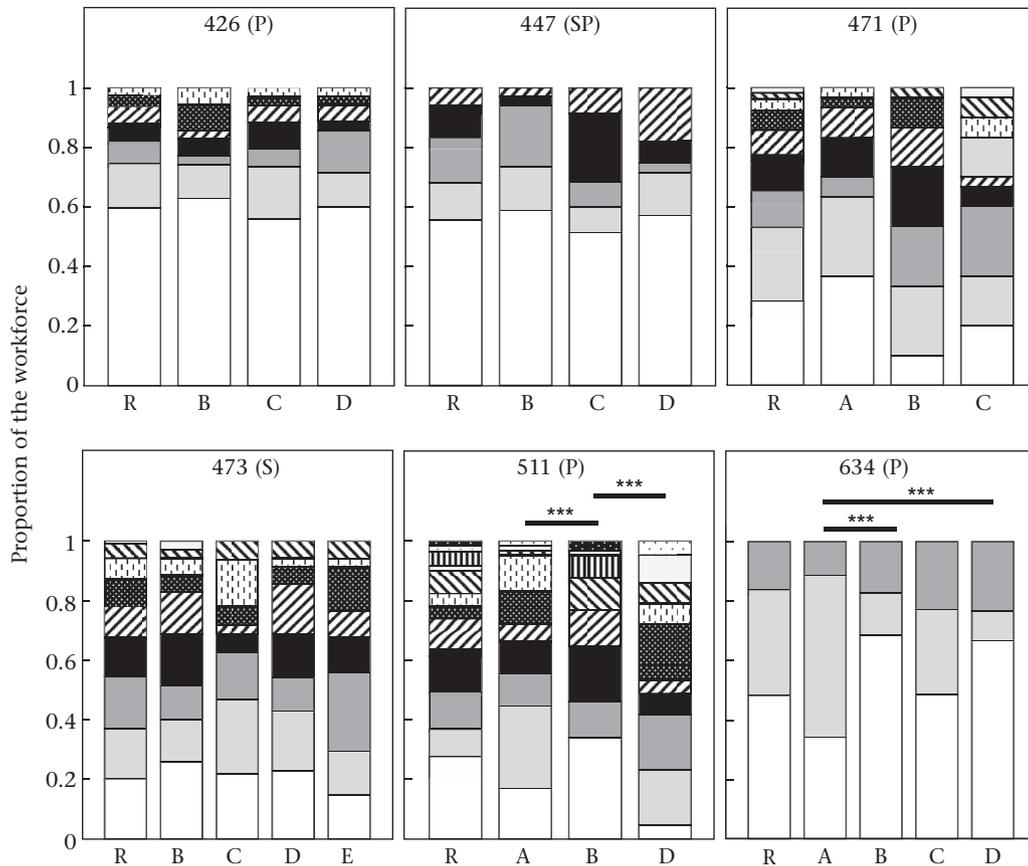
Figure 3. Stacked bar graphs showing proportions of different patrilines in the reconstructed parent colony (R) and in offspring colonies (A–E). Offspring colonies 'A' inherited the parent nest while offspring colonies 'B–E' dispersed. Differently patterned bars represent different patrilines. Asterisks indicate significance levels (corrected for multiple tests) of pairwise Fisher exact tests on patriline distributions among sister colonies (i.e. excluding reconstructed parent colonies): **$P < 0.01$; ***$P < 0.001$. Types of gynes produced are indicated following colony number (S = sexually produced, P = parthenogenetically produced, SP = both forms present). Data for reconstructed parent colonies (R) were taken as the median number of workers of each patriline over the 1000 simulated colonies (see Methods).

argue here that interindividual differences in dispersal propensity provide the most parsimonious explanation for the majority of the patterns observed for reasons explained below.

Much of the bias in allocation among sister colonies was related to worker size, with larger workers more frequently found in offspring colonies that had dispersed. Larger workers are likely to be more highly valued because they are more costly to produce (Hölldobler & Wilson, 1990; Oster & Wilson, 1978), and because of the benefits of large size (Cerdá, 2001; Heinze et al., 2003; Porter & Tschinkel, 1985). In *Cataglyphis* ants, larger workers are able to withstand higher temperatures (Clémencet et al., 2010) and have higher immune levels (Bocher, Tirard, & Doums, 2007), which may provide important foraging advantages (Cerdá, 2001; Cerdá & Retana, 1997). These advantages may be particularly important to dispersing colonies, which could gain a two-fold benefit: starting with sufficient large workers to constitute an accomplished work-force for nest excavation and foraging (which may be crucial for the rapid development of dispersing colonies and for scramble competitors as in *C. cursor*) and circumventing the cost of the production of large workers (and thereby gaining a head start towards colony development). At the same time, the loss of predominantly large workers may represent an acceptable cost to the offspring colony that inherits the parent nest, both because of the dispro-portionate advantage conferred to dispersing sister colonies and because the inheriting colony does not have to excavate a nest and is hence better positioned to manufacture new large workers to replace those lost. Alternatively, colonies may simply have no

control over which workers are allocated to which offspring colony. Worker size can provide the basis for a distribution bias if trans-porters preferentially select larger ants and/or larger ants show a higher dispersal propensity. In the latter case this could simply arise from the fact that larger ants are more prone to forage, defend and maintain the nest and therefore more likely to be found in the chambers near the entrance. Both of these scenarios can explain the allocation bias in large workers to dispersing colonies relative to inheriting colonies, although dispersal propensity has the advan-tage of not requiring us to invoke active discrimination abilities in transporting workers and avoids additional complications such as delays generated by a selection process each time transporters re-turn to the source nest, and a potential trade-off between finding an optimal target and search time.

In two of six cases, patrilines were not randomly distributed among sister colonies and, as for the distribution of worker size, this pattern could arise if workers of different patrilines exhibit different dispersal propensities. In line with previous studies on social insects (Heinze et al., 1997; Rangel et al., 2009; Solís et al., 1998), we found no evidence of nepotism during fission in the form of a greater bias in colonies containing sexually produced gynes, and indeed, the two colonies in which significant differences in the distribution of patrilines were detected contained only parthenogenetically produced gynes. Furthermore, in the two cases in which the parent colony produced gynes sexually, we found no evidence of workers being overrepresented in offspring colonies containing gynes of their own patriline, or higher bias in sister

colonies than in colonies containing only parthenogenetically produced gynes. Patriline bias may arise from one additional source in *C. cursor* regardless of the type of gyne present, although at present it is difficult to estimate its importance. Workers in *C. cursor* can produce new gynes via thelytoky in the event of colony orphaning, and abundant patrilines are more likely to mother replacement gynes (e.g. Chéron, Monnin et al., 2011). Workers of the same patrilines could thus benefit from initiating new colonies together if the probability of queen replacement was sufficiently high, although at present the frequency of queen replacement in the field is unknown. We did find that patrilines were not randomly distributed among sister colonies in one case in which all gynes were parthenogenetically produced (colonies 511A, B and D). In this case, workers of four patrilines that appear to have been mothered by a previous mother queen were absent from the colony containing the current mother queen. This may indicate allocation of individuals along kin lines, although genetic evidence also suggested a mother queen turnover had occurred in the paternal colony, and it is difficult to anticipate what influence this may have had on the fission process. Thus, while differing dispersal propensities between patrilines are sufficient to explain the majority of the genetic patterns observed during colony fission, we cannot rule out a possible occasional role of nepotism in the case of queen turnover.

Our combined results thus indicate that although we cannot rule out a role of active discrimination by transporters and occasional nepotistic behaviour, the majority of the allocation bias observed among offspring colonies can be explained by differing dispersal propensities between workers based on size and, in some cases, patriline. We stress, however, that our genetic data comprise only six mother colonies, reconstructed from the 20 offspring colonies they fissioned into, and should be treated accordingly with some caution. Previous studies have suggested that patrilineal differences in dispersal propensity can explain distribution patterns during fission in honeybees (Estoup, Solignac, & Cornuet, 1994; Getz, Brückner, & Parisian, 1982; Kryger & Moritz, 1997), and the one study that demonstrated evidence of assortative distribution of workers along kin lines during fission in ants (Seppä et al., 2008) could not rule out this possibility. Workers of different patrilines are known to vary in their tendency to perform various tasks in *C. cursor* (Eyer et al., 2013) and it seems reasonable to assume that this also applies to dispersal. However, no previous study, to our knowledge has provided evidence of size-based variation in dispersal propensity during fission. Although size varied with patriline in this study, patriline explained only 8% of the variation in worker size and this is insufficient to account for the strong morphometric patterns observed among sister colonies.

Our findings have implications for our understanding of the evolution and ecology of reproductive and mating strategies. By comparing the reconstructed parent colony and offspring colonies, we showed that fission can decrease both morphological and genetic diversity in offspring colonies. This may represent an inherent cost of fission given the benefits of a diverse workforce in social insects (e.g. Baer & Schmid-Hempel, 1999; Cerdá & Retana, 1997; Hughes & Boomsma, 2004; Reber, Castella, Christe, & Chapuisat, 2008; Ugelvig et al., 2010). The reduction in worker genetic diversity resembles that associated with a population genetic bottleneck (Nei, Maruyama, & Chakraborty, 1975). However, it is likely to be short-lived relative to a genetic bottleneck because (1) the genetic diversity of the breeding population (i.e. queens) is unaffected by fission, and thus diversity will be rapidly reinjected by the production of new brood through multiple mating and (2) brood items transported to the new site during fission are presumably exempt from bias, and will thus help restore diversity on maturity. Furthermore, qualitative investment bias may act to

erode or augment any quantitative bias in investment, and both these components may need to be incorporated into investment strategy models (e.g. Crozier & Pamilo, 1996; Pamilo, 1991) to properly understand reproductive investment under DCF. We close with one caveat to the above discussion in that age is likely to be an important factor in determining worker value, and could potentially influence worker allocation patterns and dispersal propensity during fission. However, it is at present not possible to reliably age ants and this question must remain open. None the less, because ants do not grow after eclosion as adults (Hölldobler & Wilson, 1990) any effect of age is unlikely to influence the size-linked effects we demonstrate.

Acknowledgments

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