doi:10.1111/j.1558-5646.2011.01348.x

GENETIC COMPONENTS TO CASTE ALLOCATION IN A MULTIPLE-QUEEN ANT SPECIES

Romain Libbrecht,[1,3] Tanja Schwander,[2] and Laurent Keller[1]

[1] *Department of Ecology and Evolution, Biophore Building, University of Lausanne, CH-1015 Lausanne, Switzerland*

[2] *Center for Ecological and Evolutionary studies, University of Groningen, 9700 CC Groningen, Netherlands*

[3] *E-mail: romain.libbrecht@unil.ch*

Received February 11, 2011
Accepted April 28, 2011
Data Archived: Dryad doi:10.5061/dryad.1ct56

Reproductive division of labor and the coexistence of distinct castes are hallmarks of insect societies. In social insect species with multiple queens per colony, the fitness of nestmate queens directly depends on the process of caste allocation (i.e., the relative investment in queen, sterile worker and male production). The aim of this study is to investigate the genetic components to the process of caste allocation in a multiple-queen ant species. We conducted controlled crosses in the Argentine ant *Linepithema humile* and established single-queen colonies to identify maternal and paternal family effects on the relative production of new queens, workers, and males. There were significant effects of parental genetic backgrounds on various aspects of caste allocation: the paternal lineage affected the proportion of queens and workers produced whereas the proportions of queens and males, and females and males were influenced by the interaction between parental lineages. In addition to revealing nonadditive genetic effects on female caste determination in a multiple-queen ant species, this study reveals strong genetic compatibility effects between parental genomes on caste allocation components.

KEY WORDS: **Caste determination, *Linepithema humile*, Queen specialization, sex ratio, social insects.**

One of the major transitions in evolution is the shift from solitary organisms to societies with reproductive division of labor (Maynard Smith and Szathmary 1995; Szathmary and Smith 1995). In eusocial Hymenoptera (ants, bees and wasps), reproductive division of labor is associated with morphological differences between the reproductive queens and the nonreproductive workers (Wilson 1971; Holldobler and Wilson 1990; Bourke and Franks 1995). These morphological differences, which can be extremely marked in some ant species, arise from a developmental switch during the larval stage (Wilson 1971; Holldobler and Wilson 1990).

For several decades, it was assumed that social insect female brood are fully totipotent, and that environmental factors alone determine whether an individual becomes a reproductive queen, or a functionally sterile worker. However, several recent studies have revealed that genetic factors can, and often do, play an important role in queen and worker caste determination (see Smith et al. [2008b] and Schwander et al. [2010] for review). These genetic influences range from plastic genotypes that are biased toward queen or worker development (e.g. *Pogonomyrmex rugosus* [Schwander and Keller 2008], *Acromyrmex echinatior* [Hughes and Boomsma 2008]) to a strictly genetic determination (e.g. *Pogonomyrmex* lineages [Helms Cahan et al. 2002; Julian et al. 2002; Volny and Gordon 2002], *Solenopsis xyloni* [Helms Cahan and Vinson 2003], *Wasmannia auropunctata* [Fournier et al. 2005]).

The occurrence of a genetic component to caste determination has important implications in species where colonies contain several queens, as this may influence each queen's relative reproductive success. Several studies have shown that queens within a colony may differ in their relative contribution to worker and queen production (Ross 1988; Bourke et al. 1997; Fournier et al.

2004). However, it is unknown whether these differences arise from competitive interactions among queens and other social effects or whether intrinsic genetic differences among queens and/or their mates directly bias the developmental trajectories of their female brood.

Another important factor affecting queen reproductive success in multiple-queen colonies is their relative contribution to male production. Social Hymenoptera have a haplodiploid sex determination system whereby diploid females develop from fertilized eggs whereas haploid males develop from unfertilized eggs (Crozier 1977). Both queens and workers have been shown to influence the proportion of new queens and males produced in their colonies. Queens may influence the sex ratio produced by altering the relative proportion of haploid and diploid eggs laid (Passera et al. 2001; Rosset and Chapuisat 2006) whereas workers may later affect sex ratio by selectively killing males or preferentially rearing females into queens rather than workers (Pamilo 1991; Aron et al. 1995; Passera et al. 1995; Keller et al. 1996; Sundstrom et al. 1996; Hammond et al. 2002). Accordingly, in multiple-queen colonies of ants such as *Linepithema humile*, *Pachycondyla* sp., *Pheidole pallidula* and *Formica exsecta*, queens vary in their relative contribution to male and female (queens and workers) production (Fournier and Keller 2001; Heinze et al. 2001; Fournier et al. 2004; Kummerli and Keller 2007b). Similarly, a skew in the production of males or new queens has been reported in *L. humile* and *Leptothorax acervorum* (Bourke et al. 1997; Fournier and Keller 2001). However, it remains unknown whether these contribution differences among queens have genetic components and, if so, whether these components are additive or result from epistatic and pleiotropic effects.

The aim of this study is to investigate genetic effects on the process of caste allocation (the relative investment in queen, worker and male production) in an ant species with multiple queens per colony. For this purpose, we conducted controlled crosses in the Argentine ant *L. humile.* Colonies of this species contain numerous reproductive queens (Newell 1909; Markin 1970) and, in contrast to most other ants, it is possible to obtain both males and queens, as well as induce mating, in the laboratory (Keller and Passera 1992). After conducting controlled crosses, we established single-queen colonies to study the effects of maternal and paternal genetic backgrounds, as well as the interaction between parental genomes on caste allocation.

Methods

PRODUCTION OF PARENTAL LINEAGES

We collected *L. humile* colonies on 11 February 2008 in Port-Leucate (3°2′20″E, 42°51′22″N), southern France and set up 13 single-queen colonies with 2.5 cm^3 (ca. 1000 workers). To ensure that colonies contained only brood from the mother queen, we removed all the brood present during the first two weeks. The queens were then allowed to lay eggs during eight weeks before being removed so as to stimulate the production of sexuals (new queens and males; Keller and Passera 1992, 1993). Colonies were then regularly checked to transfer all male and queen pupae to queenless and broodless recipient colonies, set up to receive the pupae of only a single sex and colony. This allowed us to obtain large numbers of unmated queens and males of the same lineage (i.e., produced by the same mother queen). These individuals were used to conduct the controlled crosses.

CONTROLLED CROSSES

Of the 26 recipient colonies, six produced enough new queens and four produced enough males to conduct replicate crosses between these maternal and paternal lineages. Mating was obtained by placing one unmated queen with four to six males overnight in a 6.5-cm-diameter vial (Keller and Passera 1992). In *L. humile*, queens are inseminated by only one male even if they mate multiple times (Keller et al. 1992; Krieger and Keller 2000). These crosses allowed us to obtain between two and eight singly inseminated queens for 22 of the 24 possible maternal-by-paternal lineage combinations (Table 1). The 110 newly mated queens were then overwintered with ca. 1000 workers for three months in the dark at 10 ± 2°C, 60% humidity to trigger the production of sexual offspring (Vargo and Passera 1992).

After overwintering, each mated queen was placed with a new set of ca. 600 workers (collected randomly in the same stock colony composed of a mix of several field colonies collected on 16 February 2009) and no brood in 20 × 14 × 5 cm transparent plastic boxes under a 12h:12h artificial light:dark cycle at 25°C, 60% humidity. Colonies were fed a mixture of mealworms, eggs, honey, and vitamins three times a week. Queens were allowed to lay eggs during six weeks before being removed. Under field conditions, 90% of the queens are killed by the workers before the beginning of the reproductive season (Markin 1970; Keller et al.

Table 1. The number of singly mated queens obtained per parental lineages combination. Each of these singly mated queens is a new queen from one of the maternal lineages (rows) inseminated by a male from one of the paternal lineages (columns).

		Paternal lineages			
		Pat1	**Pat2**	**Pat9**	**Pat13**
Maternal lineages	**Mat3**	4	6	5	-
	Mat5	4	3	8	7
	Mat6	2	4	6	4
	Mat7	-	6	2	5
	Mat8	6	6	5	8
	Mat12	2	6	5	6

1989). Thus, queen removal mimicked the conditions leading to the production of males and new queens in the field (Keller and Passera 1992, 1993). Colonies were then monitored weekly to remove all pupae produced. As the pupal stage lasts more than seven days at 25°C in *L. humile* (R. Libbrecht, pers. obs.), this allowed us to count all queen, workers, and male pupae produced, and estimate the worker/queen, male/queen, and male/female ratios.

STATISTICAL ANALYSIS

Among the 110 colonies that overwintered successfully, 20 were removed from the analysis: five queens died during the experiment, four colonies did not produce any offspring, and 11 colonies did not produce any female offspring suggesting that the queens were not inseminated. To test for the effect of maternal and paternal lineages (taken as random variables) on colony-level offspring production, we conducted two-way analyses of variance (ANOVAs) on models optimized to fit our data. The numbers of offspring, females and males were analyzed using a generalized linear model (GLM) with Poisson distributed errors. The worker/queen, male/queen, and male/female proportions were analyzed using a GLM with binomial errors. The models were checked for overdispersion and corrected when needed using quasi-likelihood to specify an appropriate variance function. Correlation tests were carried out using Spearman rank correlation tests. All statistical analyses were performed with *R* (http://www.R-project.org).

Results

Every component of caste allocation varied considerably among the single-queen colonies. Both the proportion of the female offspring that developed into queens (female caste ratio) and the proportion of queens among the sexual offspring (sex ratio) ranged from 0 to 1 (female caste ratio: 0.091 ± 0.16 and sex ratio: 0.38 ± 0.36, mean ± SD) whereas the proportion of females among all the offspring produced ranged from 0.008 to 1 (0.78 ± 0.26, mean ± SD).

For each component of caste allocation, we found significant effects of either the paternal lineage or the interaction between parental lineages. The female caste ratio was significantly influenced by the paternal lineage ($F_{3,12} = 6.44$, $P = 0.007$, Fig. 1) whereas there was no significant effect of the maternal lineage ($F_{5,12} = 1.77$, $P = 0.19$) and no significant interaction between maternal and paternal lineages ($F_{12,89} = 0.75$, $P = 0.69$). The sex ratio and the proportion of females among the offspring were not significantly influenced by the paternal (queen/male proportion: $F_{3,12} = 1.12$, $P = 0.38$; female/male proportion: $F_{3,12} = 1.82$, $P = 0.2$) or the maternal lineage (queen/male proportion: $F_{5,12} = 1.18$, $P = 0.38$; female/male proportion: $F_{5,12} = 0.70$, $P = 0.63$). By contrast, there were significant interactions between maternal and paternal lineages on both of these proportions (queen/male proportion: $F_{12,88} = 2.06$, $P = 0.032$, Fig. 2; female/male proportion: $F_{12,89} = 3.29$, $P < 0.001$, Fig. 3). The effect sizes for the different components of caste allocation are summarized in Table 2.

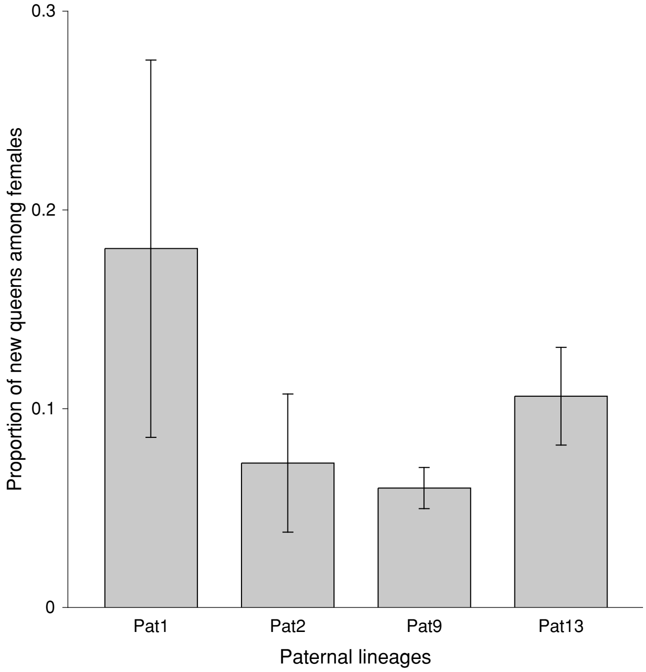

Figure 1. **The proportion of new queens among female offspring is significantly affected by the paternal lineage (mean ± SE for each paternal lineage).**

Because there appeared to be extreme variation among colonies in the total number of offspring produced (range 23–234; 120.3 ± 45.6, mean ± SD), we also tested for parental lineage effects on numbers of different offspring produced. The total number of offspring was significantly affected by the maternal lineage ($F_{5,12} = 3.33$, $P = 0.04$) but not by the paternal lineage ($F_{3,12} = 1.35$, $P = 0.30$) or by the interaction between maternal and paternal lineages ($F_{12,89} = 1.74$, $P = 0.076$). By contrast, we found a different pattern when separately analyzing the numbers of males and females produced: neither the paternal (number of males: $F_{3,12} = 1.26$, $P = 0.33$; number of females: $F_{3,12} = 2.21$, $P = 0.14$) nor the maternal lineage (number of males: $F_{5,12} = 0.81$, $P = 0.56$; number of females: $F_{5,12} = 1.52$, $P = 0.25$) significantly affected these numbers, whereas there was a significant interaction between parental lineages (number of males: $F_{12,89} = 3.24$, $P < 0.001$; number of females: $F_{12,89} = 2.45$, $P = 0.01$). The effect sizes for the numbers of male and female offspring produced are summarized in Table 2.

Finally, to test for possible allocation trade-offs, we analyzed correlations between caste numbers produced. Significant negative correlations revealed trade-offs between queen and male ($n = 90$, rho $= -0.24$, $P = 0.024$, Fig. 4), and female and male productions ($n = 90$, rho $= -0.34$, $P = 0.001$, Fig. 5). There appeared

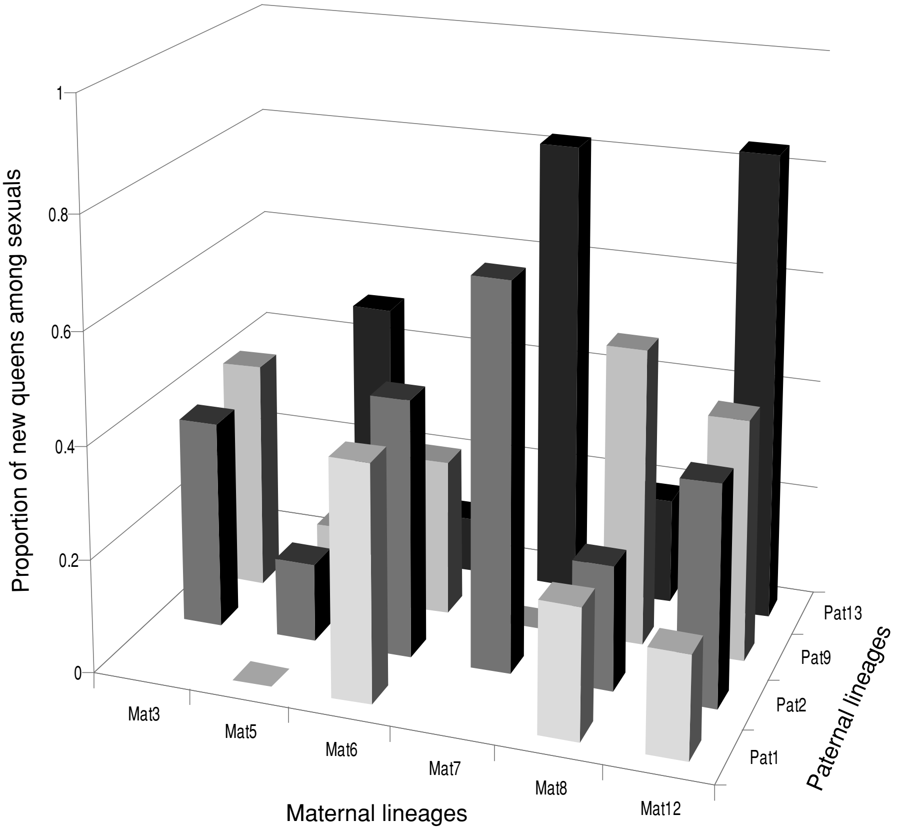

Figure 2. **The proportion of new queens among sexual offspring is significantly affected by the interaction between parental lineages (each bar depicts the mean for all queens per combination of parental lineages).**

to be no trade-off between queen and worker production as the number of queens and workers were positively correlated ($n = 90$, rho $= 0.32$, $P = 0.002$, Fig. 6).

Discussion

This study demonstrates strong effects of the maternal and paternal lineages on offspring production and caste allocation (i.e., the proportion of queens, workers and males produced) in the Argentine ant *L. humile*. The maternal lineage had a significant effect on the number of offspring produced and the paternal lineage influenced the proportion of females developing into queens or workers (i.e., the process of caste determination). There were also significant interactions between parental lineages for the two other components of caste allocation, namely the proportion of offspring being queens or males and the relative production of males and females.

Several lines of evidence suggest that these parental lineage effects have genetic components. First, all the experiments were conducted under highly controlled laboratory conditions, with colonies containing similar numbers of workers, thus largely removing possible environmental effects. Second, the workers that reared the new queens and the males were unrelated to them and all came from the same stock colonies hence ensuring a uniform social environment. Finally, after mating, queens of all lineages were placed in new colonies containing a new set of workers coming from the same stock colonies. Again, the number of workers was standardized in all colonies. As a result, mothers, fathers, and grandmothers of the broods considered in the analyses were kept under similar environmental and social conditions. This design thus makes it highly likely that parental lineage effects on caste allocation and brood production stem from genetic variation among lineages, even if some environmental influences cannot completely be ruled out. Importantly, a genetic component on sex ratio and caste allocation may stem from both direct and indirect effects. Direct influences could originate from genetic differences in offspring survival and/or development whereas indirect effects could stem from workers altering brood care and/or rearing allocations in response to changes in brood composition (Linksvayer 2006). A combination of direct and indirect effects is also possible. For example, a direct genetic effect inducing a larger proportion of females to develop into queens may reduce the resources available and lead workers to eliminate a greater proportion of males. In the following sections, we discuss in more details the effect of parental lineages on each component of offspring production and caste allocation.

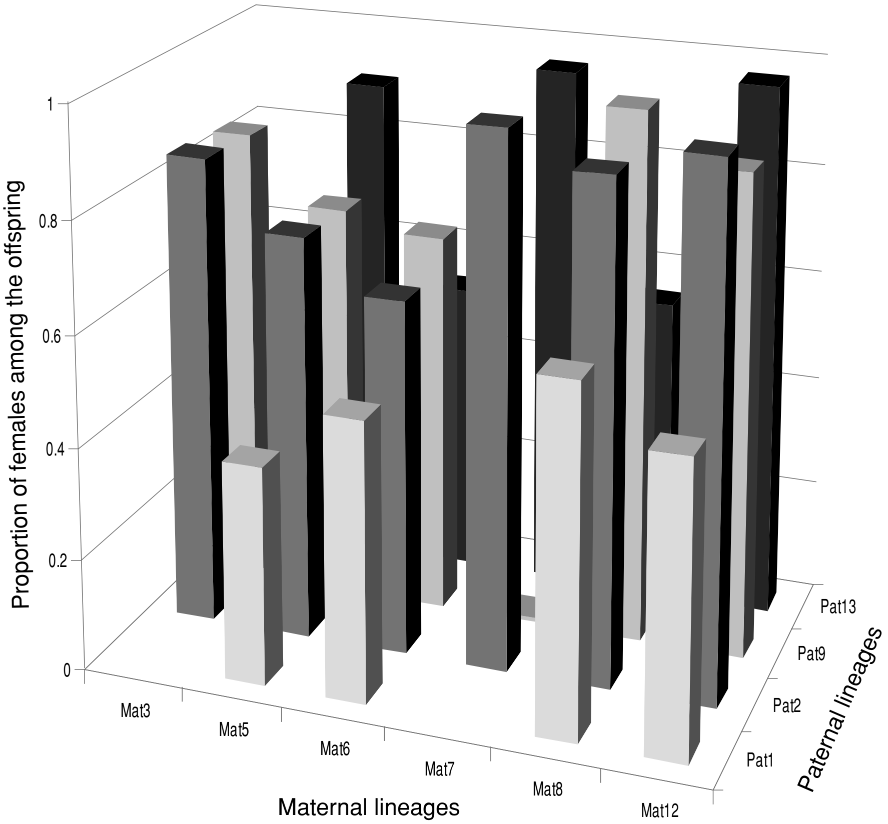

Figure 3. The proportion of females among offspring is significantly affected by the interaction between parental lineages (each bar depicts the mean for all queens per combination of parental lineages).

The first interesting finding of our study is that the paternal lineage affected the relative production of queens and workers while no significant effect of the maternal lineage was detected. This pattern reveals that the genetic effects on female caste determination in *L. humile* have a complex architecture, as classic additive effects would imply an influence of both parental lineages. This result is unlikely to stem from little statistical power for detecting maternal lineage effects. Because of haplodiploidy, the proportion of within-lineage additive genetic variation is smaller for the maternal than the paternal lineages (this is because full sisters share a larger portion of their genome than full brothers). As a consequence, additive genetic factors are more likely to generate significant effects of the maternal than the paternal lineage. The influence of only the paternal lineage on female caste fate is thus best consistent with parent of origin-specific effects and/or other epigenetic factors. Thus, caste-biasing genes could be expressed in the female brood only if paternally inherited. Alternatively, heritable epigenetic changes that affect the likelihood for the female offspring to develop into queens could be triggered by male-dependent conditions.

Table 2. Reduction of deviance obtained when the maternal lineage, the paternal lineage, or the interaction between parental lineages is added to the model. These percentages thus represent the extent to which an explanatory variable improves the model's ability to account for the empirical data. The significance of maternal and paternal lineage effects and of their interaction is also notified (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

	Maternal lineage (%)	Paternal lineage (%)	Interaction between maternal and paternal lineages (%)
Relative proportion of new queens and workers	7.08	15.43**	9.58
Relative proportion of new queens and males	11.12	6.35	22.68*
Relative proportion of females and males	7.86	12.24	26.9***
Number of offspring	22.48*	5.46	16.18
Number of females	13.09	11.46	20.68**
Number of males	9.68	8.99	28.49***

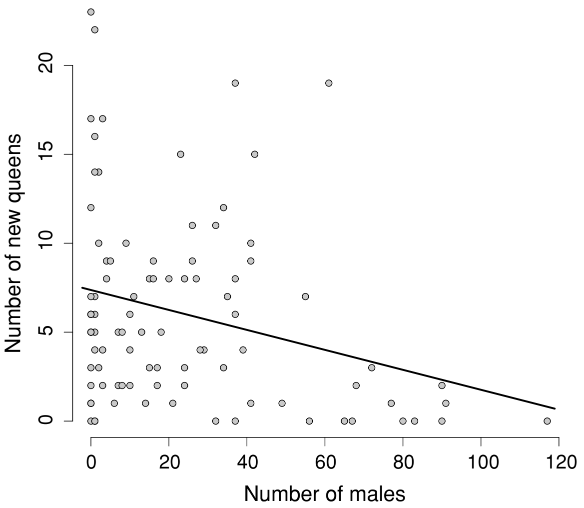

Figure 4. **Across colonies the number of new queens produced is negatively correlated with the number of males produced (Spearman rank correlation test; $n = 90$, rho $= -0.24$, $P = 0.024$).**

One important issue discussed by previous studies that reported genetic effects on female caste determination is the maintenance of genetic variation for the trait, as alleles biasing caste development toward queens should quickly go to fixation (Crozier and Pamilo 1996). Four hypotheses have been proposed to account for the maintenance of genetic variation. First queen-biasing alleles are associated with costs such as decreased colony productivity (Bourke and Ratnieks 1999; Wenseleers and Ratnieks 2004). Second, queen-biasing alleles are deleterious when in the homozygous form (Keller and Ross 1998; Hayashi et al. 2007). Third, under sexual antagonism (Rice 1984), queen-biasing alleles, which are favored in females, decrease male fitness (Moritz et al. 2005). Finally, genetic influences on female caste can be maintained if the genetic architecture underlying caste biasing is complex (Schwander et al. 2010). The finding of imprinting and/or epigenetic effects on caste determination is very interesting in this perspective because it reveals a new type of genetic influences that are more complex than additive genetic effects. The traditional method used to test for genetic components to caste determination is to compare the relative representations of patrilines among new queens and workers in species with only one multiply mated queen per colony (Hughes and Boomsma 2008; Schwander and Keller 2008; Smith et al. 2008a; Frohschammer and Heinze 2009). However, this experimental design does not allow inferring the genetic architecture underlying caste bias. We call for more studies on the influence of genetic architecture on the developmental fate of female brood to get a better understanding of the maintenance of genetic effects on caste determination in social insect species.

Our study also revealed significant interactions between parental lineages on sex ratio (proportion of queens and males) while neither the maternal nor the paternal lineage affected this proportion. The lack of maternal and paternal lineage effects are difficult to interpret given that the source colonies used for setting up the parental lineages already showed a biased sex ratio (male bias for the paternal and queen bias for the maternal lineages). This may have altered the distribution of genetic variability for sex ratio between the maternal and paternal lineages. By contrast, several mechanisms could explain the interaction between parental lineages on sex ratio. Sex ratio could be influenced directly by interactions between parental lineages if compatibility between parental genomes affects the viability of female broods or influences the likelihood of egg fertilization. Alternatively, sex ratio may be influenced indirectly via changes in the proportion of females that develop into queens rather than workers. The latter

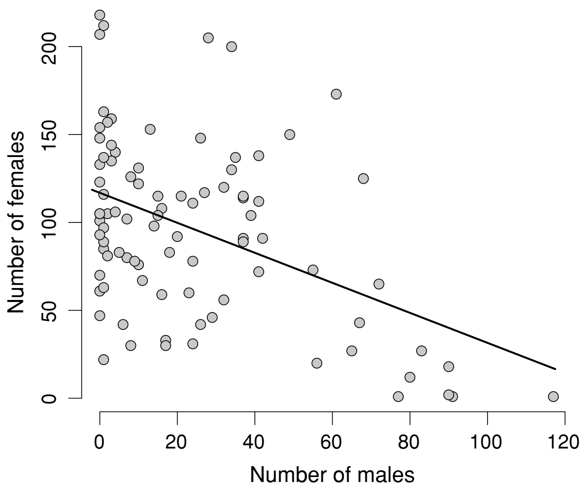

Figure 5. **Across colonies the number of females produced is negatively correlated with the number of males produced (Spearman rank correlation test; $n = 90$, rho $= -0.34$, $P = 0.001$).**

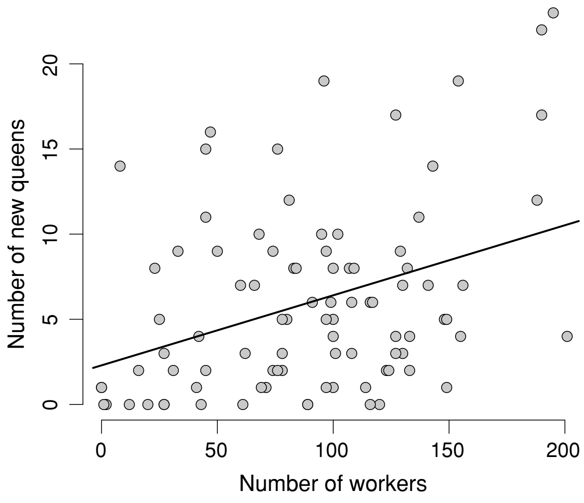

Figure 6. **Across colonies the number of new queens produced is positively correlated with the number of workers produced (Spearman rank correlation test; $n = 90$, rho $= 0.32$, $P = 0.002$).**

explanation is unlikely in *L. humile* because, contrary to the proportion of males and females, the proportion of new queens and workers produced was not significantly affected by the interaction between parental lineages. Our analyses of brood numbers are best consistent with compatibility affecting fertilization probability, although additional effects on female brood viability or the worker propensity to preferentially raise new queens and/or eliminate males remain possible. Indeed, the numbers of males and females produced were negatively correlated and both were significantly affected by interactions between the maternal and paternal lineages. This is the expected pattern if queens laid a fixed number of eggs independently of the ratio of haploid to diploid eggs among them, thereby generating a trade-off in the numbers of males and diploid brood produced. Queens may actively change sex allocation depending on qualities of the sperm transferred by their mate (Fjerdingstad and Boomsma 1997; Fjerdingstad 2004) or fertilization success may be passively influenced by compatibilities between parental genomes. Whatever the detailed mechanism, our results reveal that interactions between queens and males can affect the colony sex ratio. Previous studies showed that the queen influences the colony sex ratio in *S. invicta*, *F. selysi*, and *Cardiocondyla kagutsuchi* (Passera et al. 2001; Rosset and Chapuisat 2006; Frohschammer and Heinze 2009). However, because these studies were not designed to detect potential effects of the interaction between the queens and their mates, it is impossible to infer whether the reported queen influences also stem from interaction effects or between-queen differences.

The finding that interactions between the queen and her mate may affect the relative production of new queens and males has important implications for sex ratio and conflict theory in social insects. Because of the haplodiploid mechanism of sex determination, there is a potential conflict between queens and their mates over the sex ratio produced (Haig 1998; Helantera and Ratnieks 2009), as males have all their genes in their daughters but none in the males produced. There is thus strong pressure on males to bias the sex ratio toward females (Haig 1998) while queens should favor balanced sex ratios because they are equally related to their daughters and sons. The finding that the interaction between queens and males can influence the sex ratio produced should be added to the traditional queen/worker framework when studying intracolonial conflicts in social insects.

Our study also provides a new explanation for why queen in multiqueen societies often tend to specialize in the production of a single caste. For example, queens producing more males produce fewer queens in *L. humile* (Fournier and Keller 2001) and *F. exsecta* (Kummerli and Keller 2007a, b). A trade-off between the contribution to worker and queen production has been reported in *P. pallidula* (Fournier et al. 2004) and a trade-off between worker and male production in *L. acervorum* (Hammond et al. 2006). In these studies, it was not possible to determine whether the queen specializations (Kummerli and Keller 2007a,b) resulted from competition and social interactions between queens or from intrinsic differences between the broods produced by queens. In our single-queen colonies, we found similar trade-offs for the relative investment into males and queens, as well as males and females, revealing that competition and social interactions between queens are not required to generate specializations. In addition, given that caste allocation in our colonies was also influenced by the interaction between parental lineages, queen specialization reported in the previous studies may at least partly stem from genetic differences. More generally, queen specialization is likely to be affected by complex genetic interactions between the queen and her mate. Such interaction effects between queens and males may help to explain the maintenance of queen specialization in social insect species with multiple queens per colony.

Finally, we also found that the total number of offspring produced in our experimental colonies was affected exclusively by the maternal lineage. This is not surprising given that both the number of eggs produced and the nutrients in the eggs depend on the mother queens. Because males only contribute their sperm to offspring production, paternal effects in this case would have to occur mainly via some type of chemical manipulation of the females, possibly in combination with effects on diploid brood viability. The effect of the maternal lineage on the number of offspring produced thus stems most likely from between-lineage variation in fecundity and/or egg viability. This variation may derive from genetic differences between queens from different lineages (Frohschammer and Heinze 2009), maternal effects (Schwander et al. 2008), and/or different environmental conditions experienced by different lineages during their development. As explained above, all colonies were maintained under highly controlled conditions so that differences in environmental conditions should only have a minor contribution to differences between lineages compared to genetic or maternal effects.

In conclusion, the use of controlled crosses in the laboratory allowed us to demonstrate widespread effects of both parental genetic backgrounds on several components of caste allocation. Our study provides evidence that nonadditive genetic effects account for between-queen and between-colony variations in the caste and sex ratios produced. Such diverse influences of nonadditive genetic effects demonstrate overall complex architectures of the genetic components to caste allocation. More such studies are strongly needed to develop insights into the genetics of phenotypic plasticity and caste allocation in social insects.

ACKNOWLEDGMENTS

We thank C. Tanner and two anonymous reviewers for their helpful comments on the manuscript. This research was supported by several grants from the Swiss National Science Foundation and an ERC advanced grant.

LITERATURE CITED

Aron, S., E. L. Vargo, and L. Passera. 1995. Primary and secondary sex-ratios in monogyne colonies of the fire ant. Anim. Behav. 49:749–757.

Bourke, A. F. G., and N. R. Franks. 1995. Social evolution in ants. Princeton Univ. Press, Princeton, NJ.

Bourke, A. F. G., H. A. A. Green, and M. W. Bruford. 1997. Parentage, reproductive skew and queen turnover in a multiple-queen ant analysed with microsatellites. Proc. R. Soc. Lond. B 264:277–283.

Bourke, A. F. G., and F. L. W. Ratnieks. 1999. Kin conflict over caste determination in social Hymenoptera. Behav. Ecol. Sociobiol. 46:287–297.

Crozier, R. 1977. Evolutionary genetics of the Hymenoptera. Annu. Rev. Entomol. 22:263–288.

Crozier, R. H., and P. Pamilo. 1996. Evolution of social insect colonies, sex allocation and kin-selection. Oxford Univ. Press, Oxford.

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

Fjerdingstad, E. J., and J. Boomsma. 1997. Variation in size and sperm content of sexuals in the leafcutter ant *Atta colombica.* Insectes Soc. 44: 209–218.

Fournier, D., and L. Keller. 2001. Partitioning of reproduction among queens in the Argentine ant, *Linepithema humile*. Anim. Behav. 62: 1039–1045.

Fournier, D., S. Aron, and L. Keller. 2004. Significant reproductive skew in the facultatively polygynous ant *Pheidole pallidula*. Mol. Ecol. 13: 203–210.

Fournier, D., A. Estoup, J. Orivel, J. Foucaud, H. Jourdan, J. Le Breton, and L. Keller. 2005. Clonal reproduction by males and females in the little fire ant. Nature 435:1230–1234.

Frohschammer, S., and J. Heinze. 2009. A heritable component in sex ratio and caste determination in a *Cardiocondyla* ant. Front. Zool. 6:27.

Haig, D. 1998. Mother's boy or daddy's girl? Sex determination in Hymenoptera. Trends Ecol. Evol. 13:380–381.

Hammond, R. L., M. W. Bruford, and A. F. G. Bourke. 2002. Ant workers selfishly bias sex ratios by manipulating female development. Proc. R. Soc. Lond. B 269:173–178.

———. 2006. A test of reproductive skew models in a field population of a multiple-queen ant. Behav. Ecol. Sociobiol. 61:265–275.

Hayashi, Y., N. Lo, H. Miyata, and O. Kitade. 2007. Sex-linked genetic influence on caste determination in a termite. Science 318:985–987.

Heinze, J., B. Trunzer, B. Holldobler, and J. H. C. Delabie. 2001. Reproductive skew and queen relatedness in an ant with primary polygyny. Insectes Soc. 48:149–153.

Helantera, H., and F. Ratnieks. 2009. Sex allocation conflict in insect societies: who wins? Biol. Lett. 5:700.

Helms Cahan, S., J. D. Parker, S. W. Rissing, R. A. Johnson, T. S. Polony, M. D. Weiser, and D. R. Smith. 2002. Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. Proc. R. Soc. Lond. B 269:1871–1877.

Helms Cahan, S. H., and S. B. Vinson. 2003. Reproductive division of labor between hybrid and nonhybrid offspring in a fire ant hybrid zone. Evolution 57:1562–1570.

Holldobler, B., and E. Wilson. 1990. The ants. Belknap press of Harvard University Press, Cambridge, MA.

Hughes, W., and J. Boomsma. 2008. Genetic royal cheats in leaf-cutting ant societies. Proc. Natl. Acad. Sci. USA 105:5150.

Julian, G. E., J. H. Fewell, J. Gadau, R. A. Johnson, and D. Larrabee. 2002. Genetic determination of the queen caste in an ant hybrid zone. Proc. Natl. Acad. Sci. USA 99:8157–8160.

Keller, L., and L. Passera. 1992. Mating system, optimal number of matings, and sperm transfer in the Argentine ant *Iridomyrmex humilis*. Behav. Ecol. Sociobiol. 31:359–366.

———. 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.

Keller, L., and K. Ross. 1998. Selfish genes: a green beard in the red fire ant. Nature 394:573–575.

Keller, L., S. Aron, and L. Passera. 1996. Internest sex-ratio variation and male brood survival in the ant *Pheidole pallidula*. Behav. Ecol. 7:292.

Keller, L., L. Passera, and J. P. Suzzoni. 1989. Queen execution in the Argentine ant, *Iridomyrmex humilis*. Physiol. Entomol. 14:157–163.

Keller, L., L. Passera, and D. Cantoni. 1992. Repeated mating by queens results in a single insemination in the Argentine ant *Iridomyrmex humilis*. Pp. 35–39 *in* Biology and evolution of social insects. Leuven Univ. Press, Leuven, Belgium.

Krieger, M. J. B., and L. Keller. 2000. Mating frequency and genetic structure of the Argentine ant *Linepithema humile*. Mol. Ecol. 9:119–126.

Kummerli, R., and L. Keller. 2007a. Extreme reproductive specialization within ant colonies: some queens produce males whereas others produce workers. Anim. Behav. 74:1535–1543.

Kummerli, R., and L. Keller. 2007b. Reproductive specialization in multiple-queen colonies of the ant *Formica exsecta*. Behav. Ecol. 18:375–383.

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

Markin, G. 1970. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in southern California. Ann. Entomol. Soc. Am. 63:1238–1242.

Maynard Smith, J., and E. Szathmary. 1995. The major transitions in evolution. Oxford Univ. Press, Oxford, UK.

Moritz, R., H. Lattorff, P. Neumann, F. Kraus, S. Radloff, and H. Hepburn. 2005. Rare royal families in honeybees, *Apis mellifera*. Naturwissenschaften 92:488–491.

Newell, W. 1909. The life history of the Argentine ant. J. Econ. Entomol. 2:174–192.

Pamilo, P. 1991. Evolution of colony characteristics in social insects. I. Sex allocation. Am. Nat. 137:83.

Passera, L., S. Aron, and D. Bach. 1995. Elimination of sexual brood in the Argentine ant *Linepithema humile*—Queen effect and brood recognition. Entomol. Exp. Appl. 75:203–212.

Passera, L., S. Aron, E. Vargo, and L. Keller. 2001. Queen control of sex ratio in fire ants. Science 293:1308.

Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742.

Ross, K. 1988. Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 23:341–355.

Rosset, H., and M. Chapuisat. 2006. Sex allocation conflict in ants: when the queen rules. Curr. Biol. 16:328–331.

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

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

Schwander, T., N. Lo, M. Beekman, B. Oldroyd, and L. Keller. 2010. Nature versus nurture in social insect caste differentiation. Trends Ecol. Evol. 25:275–282.

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

Smith, C., A. Toth, A. Suarez, and G. Robinson. 2008b. Genetic and genomic analyses of the division of labour in insect societies. Nat. Rev. Genet. 9:735–748.

Sundstrom, L., M. Chapuisat, and L. Keller. 1996. Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. Science 274:993.

Szathmary, E., and J. M. Smith. 1995. The major evolutionary transitions. Nature 374:227–232.

Vargo, E. L., and L. Passera. 1992. Gyne development in the Argentine ant *Iridomyrmex humilis*—role of overwintering and queen control. Physiol. Entomol. 17:193–201.

Volny, V. P., and D. M. Gordon. 2002. Genetic basis for queen-worker dimorphism in a social insect. Proc. Natl. Acad. Sci. USA 99:6108–6111.

Wenseleers, T., and F. Ratnieks. 2004. Tragedy of the commons in *Melipona* bees. Proc. R. Soc. Lond. B 271:S310.

Wilson, E. O. 1971. The insect societies. Harvard Univ. Press, Cambridge, MA.

Associate Editor: J. Strassmann