Insect. Soc.
DOI 10.1007/s00040-017-0556-9

Insectes Sociaux

RESEARCH ARTICLE

Frequent colony orphaning triggers the production of replacement queens via worker thelytoky in a desert-dwelling ant

Fernando Amor[1] · Patrocinio Ortega[1] · Raphaël Boulay[2] · Xim Cerdá[1]

Received: 27 October 2016 / Revised: 14 February 2017 / Accepted: 2 March 2017

Abstract Although related, *Cataglyphis floricola* and *Cataglyphis tartessica* show very different responses to colony orphaning. In the laboratory, under queenless conditions, *C. tartessica* workers produced male offspring via arrhenotoky, while *C. floricola* workers produced female offspring, including new queens, via thelytoky. Both species have workers with active ovaries that produce trophic eggs. In the field, in the late spring, *C. floricola* colonies were more likely to be orphaned than were *C. tartessica* colonies, probably due to differences in how they performed fission, their colony foundation system. The combined action of these two features could explain the presence of thelytoky in *C. floricola* and its absence in *C. tartessica*.

Keywords Orphaning · Thelytoky · Desert ant · Trophic eggs · Fission · *Cataglyphis floricola* · *Cataglyphis tartessica*

Introduction

Parthenogenesis is a form of asexual reproduction in which offspring are produced by a single parent (i.e. without gamete fertilisation). It allows individuals to transfer a carbon copy of their genomes to the next generation. However, the main drawback of parthenogenesis, as compared to sexual reproduction, is the inability, or at least very low ability, depending on the degree of recombination, to respond evolutionarily to environmental variability (Smith 1971; Rispe et al. 1998). In Hymenoptera, the standard method is arrhenotokous parthenogenesis, in which males are produced from unfertilised haploid eggs. The dynamics of arrhenotoky are of special interest in eusocial species, which display a reproductive division of labour between queens and workers. Workers lack spermatheca, which makes them unable to reproduce sexually (Hölldobler and Wilson 1990). However, in many species, workers do have ovaries and thus can reproduce asexually via arrhenotoky. Yet, workers generally only reproduce when the colony lacks a fertile queen (Hammond and Keller 2004).

In contrast, thelytokous parthenogenesis leads to the production of diploid females. Thelytoky has frequently been observed in the ant genus *Cataglyphis* (Rabeling and Kronauer 2013; Cronin et al. 2016; Boulay et al. 2017). For example, queens produce new queens via thelytoky in *C. cursor* (Pearcy et al. 2004; Doums et al. 2013a), *C. hispanica* (Leniaud et al. 2012), and *C. velox* (Eyer et al. 2013); in contrast, workers come from fertilised eggs. Worker thelytoky has been seen in queenless laboratory colonies of *C. cursor* (Cagniant 1979), *C. sabulosa* (Timmermans et al. 2008), and *C. savignyi* (Leniaud et al. 2011). While it often yields sterile diploid males, it may also result in the production of new queens. Consequently, if a colony finds itself in the hopeless situation of having been orphaned, one of the new queens may be able to mate with a male from outside the colony and thus replace her mother.

In this study, we compared worker reproduction in two species of desert ants: *Cataglyphis floricola* and *Cataglyphis tartessica* (Fig. 1a, b). Until very recently, the two

---

Electronic supplementary material The online version of this article (doi:10.1007/s00040-017-0556-9) contains supplementary material, which is available to authorized users.

✉ Fernando Amor
fernandoamor01@gmail.com

[1] Estación Biológica de Doñana, CSIC, Seville, Spain

[2] Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François Rabelais, Tours, France

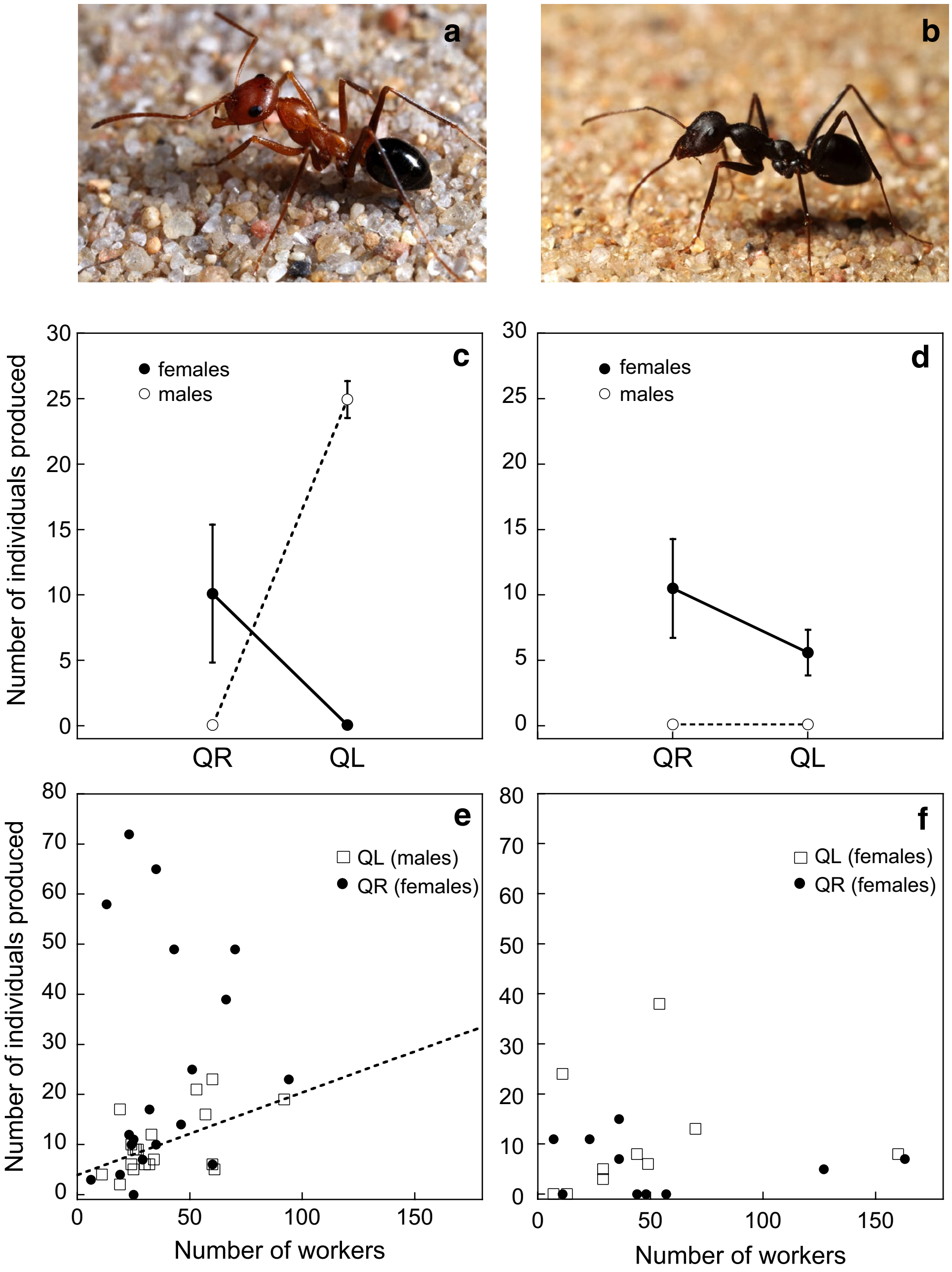

Fig. 1 Offspring production in *C. tartessica* and *C. floricola*. Workers of *C. tartessica* (**a**) and *C. floricola* (**b**). Mean number (±SE) of females (*black dots, plain lines*) and males (*white dots, dotted lines*) in queenright (QR) and queenless (QL) groups of *C. tartessica* (**c**) and *C. floricola* (**d**). Number of individuals produced in (**e**) *C. tartessica* QR (*plain symbols*—females) and QL groups (*open symbols*—males) and in (**f**) *C. floricola* QR (*plain symbols*—females) and QL groups (*open symbols*—females)

were considered to be different morphs of the same species. However, recent molecular analyses have revealed that gene flow between them ended about 1 million years ago, providing evidence for their status as two distinct species (Jowers et al. 2014; Amor and Ortega 2014). Both are monogynous and nearly monandrous. They disperse by fission. In the case of *C. tartessica*, a mother colony splits to produce one daughter colony and, on rare occasions, two (Amor et al. 2011b). Whether colony fission in *C. floricola* follows the same pattern is unknown. Because the ants disperse on foot, the queens' wings have become non-functional: both species produce flightless queens. There are two different queen morphs: brachypters and ergatoids. The former sheds its short, non-functional wings just after mating, while the latter is permanently wingless (Amor et al. 2011b).

Materials and methods

Between 2010 and 2014, we collected 10 colonies of *C. floricola* and 19 colonies of *C. tartessica* from Doñana National Park (southwestern Spain). They were brought to the laboratory, where the number of adults and pupae was counted. Colonies were placed in Petri dishes and had access to water and food (honey and mealworms). They were exposed to 10–14 h of natural light per day and temperatures from 15 to 30 °C, depending on the time of year. These conditions reflected those experienced by the species in the field (Amor et al. 2011a).

Each colony was divided into two groups with approximately the same number of workers; they were given no brood (i.e. no eggs, larvae, or pupae). One group contained the mother queen (queenright, QR), while the other was queenless (QL). The *C. floricola* QR and QL groups contained 46.60 ± 6.81 and 55.20 ± 7.69 workers (mean ± SE, $n = 10$), respectively. The *C. tartessica* QR and QL groups contained 37.47 ± 2.49 and 37.84 ± 2.30 workers (mean ± SE, $n = 19$), respectively. Both groups' offspring production was monitored for 1 year. Additionally, we utilised 17 colonies of *C. floricola* and 5 colonies of *C. tartessica* that were queenless upon collection.

To characterise the fission process in *C. floricola*, we mapped all the colonies found in an area of 675 m^2 (27×25 m) in May 2016 in Doñana Natural Park. The site was monitored twice a week for 1 month. We recorded the occurrence of social transport between nests, which was considered to be a sign of colony fission. Once social transport ceased, the mother and daughter colony (colonies) were excavated. The number of colony members was counted in the laboratory (Supplementary material 1). We also dissected a total of 405 *C. floricola* workers (141 workers from 7 mother colonies and 264 workers from 9 daughter colonies) and 101 *C. tartessica* workers (from 3 randomly chosen colonies). The presence of yellow bodies indicates recent egg laying (Gobin et al. 1998). In our statistical analyses, we used the presence/absence (1 vs 0) of yellow bodies.

Additionally, between 2010 and 2016, we collected, as a field study of orphaning, 26 *C. floricola* colonies and 101 *C. tartessica* colonies from Doñana National Park to assess the frequency of orphaning in nature. The number of colony members was counted in the laboratory.

We compared offspring production in *C. floricola* and *C. tartessica* under QR and QL conditions, as well as the number of developed oocytes per worker between mother and daughter colonies of *C. floricola* (i.e. colonies undergoing fission). To this end, we carried out two linear models in R (v. 2.14.1) (R Core Team 2012). Nonparametric statistical analyses were carried out using STATISTICA 12 software.

Results

In the laboratory, female offspring (workers and queens) were produced in 18 of the 19 QR groups of *C. tartessica* but not in any of the QL groups. In contrast, male offspring were produced in all the QL groups but not in any of the QR groups (Fig. 1c). The number of females produced in the QR groups ranged between 0 and 72 and did not vary with worker number (Fig. 1e; linear model: $F_{1,17} = 0.341$; $P = 0.567$). 14 of the 474 females produced in the 18 QR groups were queens (i.e. 3%); the others were workers. Only one male was produced in the QR groups. The number of males produced in the QL groups ranged between 2 and 23 and increased significantly with worker number (Fig. 1e; linear model: $F_{1,17} = 7.223$; $P = 0.016$).

C. floricola showed drastically different results: 8 of the 10 QR and QL groups produced female offspring but neither group produced male offspring (Fig. 1d). There was no correlation between the number of females produced and worker number in either group (linear model: $F_{1,8} = 0.084$; $P = 0.779$ and $F_{1,8} = 0.047$; $P = 0.833$, respectively) (Fig. 1f). Of the 105 females produced by the *C. floricola* QR groups, only 3, or 2.9%, were queens (ergatoids) (Table 1). This percentage did not differ significantly from that in the *C. tartessica* QR groups (14 queens out of 474 females; Fisher's exact test: $P = 1$). However, a higher percentage of queens was produced in *C. floricola* vs *C. tartessica* in the QL groups (15 vs 0 queens, respectively; Fisher's exact test: $P < 0.001$). These results indicate that queen removal in *C. floricola* triggered worker thelytoky, which led to the production of workers and queens. These results were confirmed by additional samples, from colonies that were queenless upon collection. The *C. tartessica* QL colonies produced 47 males and 0 females, while the *C. floricola* QL colonies produced 2 males and 250 females (more specifically 228 workers, 12 ergatoids, and 18 brachypters), a significant difference (Fisher's exact test: $P < 0.0001$).

Table 1 Production of diploid brachypters, diploid ergatoids, diploid workers, and haploid males by *C. floricola* and *C. tartessica* queenright (QR) and queenless (QL) laboratory colonies

	Cataglyphis floricola		*Cataglyphis tartessica*	
Production	QR	QL	QR	QL
Brachypterous queens	0	**6 (10.7%)**	4 (0.84%)	0
Ergatoid queens	3 (2.9%)	**6 (10.7%)**	10 (2.1%)	0
Workers	105 (97.1%)	**44 (78.6%)**	460 (96.8%)	0
Males	0	0	1 (0.3%)	192 (100%)

In *brackets* are their relative representation (i.e. percentages). In *bold* are the ants resulting from thelytoky

The field plot contained 52 nests (density: 0.08 nests/m^2). Five *C. floricola* fission events were observed. To improve statistical power, we included two fission events that happened outside the plot. These 7 events produced 14 daughter colonies [fission distance = 7.92 ± 5.28 m (mean ± SD)]. Each mother colony produced between two and five daughter colonies. On one occasion, a daughter colony immediately split to produce a second daughter colony. The seven mother colonies and eight daughter colonies were excavated, and their demographic make-up was analysed. No queen was found in any of the 15 colonies, suggesting that fission had already begun when we started our survey: more specifically, the colonies that we thought were mother colonies may actually have been produced by recent fission events. The fact that there was no difference in the size of the mother and daughter colonies supports this idea (Mann–Whitney *U* test: $Z = 0.64$; $P = 0.52$). These colonies were, however, much smaller than those collected randomly in May–June, in the field study of orphaning (worker number in fissioning colonies vs in randomly collected colonies: 40.1 ± 19.3 vs 146.3 ± 66.6, mean ± SD; Mann–Whitney *U* test: $Z = 4.68$; $P < 0.0001$). Males in a cocoon or imago phase were found in four of the seven mother colonies but not in any of the daughter colonies.

Workers with developed oocytes in their ovaries and displaying egg-laying activity were observed in both species (*C. floricola* and *C. tartessica*). In *C. floricola*, workers from the mother colonies were less likely to have developed ovaries than those from the daughter colonies (number of oocytes in ovaries of workers from mother vs daughter colonies: 2.83 ± 0.33 vs 7.78 ± 0.40, mean ± SE; linear model: $F_{1,403} = 69.2$; $P < 0.0001$). The number of workers with yellow bodies was also higher in daughter than in mother colonies (Chi-squared = 42.38; $df = 1$; $P < 0.0001$). In *C. tartessica*, and in the three randomly chosen colonies, the number of developed oocytes per worker was 8.2 ± 0.79 (mean ± SE).

The field study of orphaning revealed that, in the late spring (May–June), *C. floricola* colonies were significantly more likely to be orphaned than were *C. tartessica* colonies (Fisher's exact test: $P = 0.006$; Fig. 2). However, there was no difference between the two species in the early spring (March–April) or summer (August–September) ($P = 1$ in both cases; Fig. 2).

Discussion

Under laboratory conditions, *C. tartessica* colonies produced only females when the mother queen was present and only males when she was absent. This outcome is relatively common in ants, in which the queen lays fertilised diploid eggs that mostly develop into workers. In the queen's presence, workers remain sterile. However, if she is removed, workers engage in arrhenotoky, laying eggs that develop into males (Hammond and Keller 2004; Bourke 1988; Crozier and Pamilo 1996). The situation was very different for the *C. floricola* colonies: the workers produced daughters—new workers as well as ergatoid and brachypterous queens—via thelytoky, but no sons.

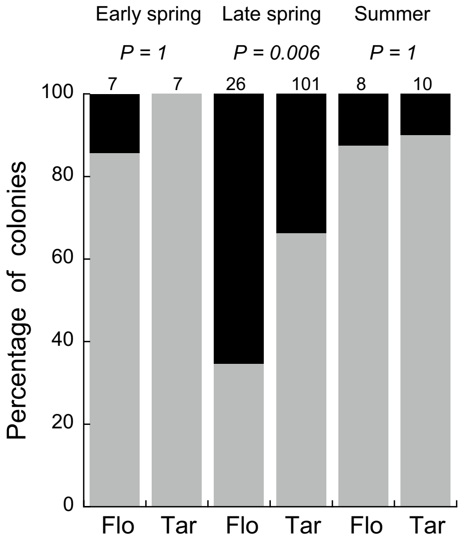

Fig. 2 Percentages of queenless (QL, *black bars*) and queenright (QR, *grey bars*) colonies of *C. floricola* and *C. tartessica* collected in the early spring (March–April), late spring (May–June), and summer (August–September). The numbers at the tops of the bars indicate the number of replicates (*n*). The two species differed significantly in their percentages of queenless vs queenright colonies in the late spring (Fisher's exact test)

However, the question arises: why was worker thelytoky present under queenless conditions in the *C floricola* colonies but absent in the *C. tartessica* colonies? Both species have diverged very recently (maybe no later than 1 million years ago) which suggests that the occurrence of thelytoky has a low phylogenetic constrain. Otherwise, both species seem to have workers that lay trophic eggs, which help distribute nutrients to nestmates; this is a unique characteristic of social insects that is especially common in ants (Hölldobler and Wilson 1990). In addition, workers with active ovaries may be able to quickly produce reproductive eggs after queen loss (West-Eberhard 1981). Our results show that, at least in *C. floricola*, these workers are transported to new daughter nests. We argue that thelytoky's benefits may be dependent on the likelihood of being orphaned; according to our field data, the likelihood of orphaning in the late spring was greater for *C. floricola* than for *C. tartessica*. The reason for this could lie in differences in how the two species carry out fission. The larger number of daughter colonies produced by *C. floricola* could results in an increased probability of remaining isolated from the mother colony and, consequently, becoming orphans.

To answer the question regarding the absence of thelytoky in *C. tartessica*, we must also consider the potential costs associated with this process. One of these costs is the production of sterile diploid males (Agoze et al. 1994; but see Cowan and Stahlhut 2004, Doums et al. 2013a). In hymenopterans, an individual develops as a female only when it is heterozygous at the sex locus, while hemizygous or homozygous state determines fertile haploid male or sterile diploid male respectively (van Wilgenburg et al. 2006). The production of diploid males may be a high cost on colony fitness because (1) they are usually sterile or they father sterile, triploid female progeny, and (2) queens mated with a single male carrying the same sex allele will produce 50% diploid males instead of workers (Pearcy et al. 2009). Additional costs associated with thelytoky are the lower success of thelytokous egg hatching (Miyakawa and Mikheyev 2015a), the smaller size of new queens and the possibility of social conflicts among workers regarding the parentage of new queens (Doums et al. 2013a; Greeff 1996). Finally, thelytoky may increase the cost of inbreeding (Doums et al. 2013b). Note, however, that in a few species with unorthodox reproductive systems, queen thelytoky allows separating male and female genomes and thus prevents inbreeding (Pearcy et al. 2011; Miyakawa and Mikheyev 2015b). As a result, it may be that, for *C. tartessica*, the previous costs of worker thelytoky do not outweigh its potential benefits (Tsuji 1995; Teseo et al. 2013).

Our study suggests that thelytokous production of workers and queens by queenless workers of *C. floricola* could be an adaptive response to the higher probability of colony orphaning in the late spring. This process could be facilitated by the presence of workers with developed and active ovaries. Moreover, newly produced thelytokous queens could subsequently copulate with males that emerge during the male production period (June–July; Amor et al. 2011a) and thus ensure colony survival. Future research should quantify thelytokous queen production in *C. floricola* and its impact on population genetic structure.

Acknowledgements We thank Sara Castro and Benjamin Carbonne for their help in the field. Fieldwork facilities were provided by ICTS-RBD. Ant collection procedures and experiments comply with Spanish legislation. This study was funded by the Spanish Ministry of Economy and Competitiveness and FEDER (projects CGL2012-36181 and CGL2015-65807).

References

Agoze M, Drezen JM, Renault S, Periquet G (1994) Analysis of the reproductive potential of diploid males in the wasp *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). Bull Ent Res 84:213–218

Amor F, Ortega P (2014) *Cataglyphis tartessica* sp.n., a new ant species (Hymenoptera: Formicidae) in south-western Spain. Myrmecol News 19:125–132

Amor F, Ortega P, Cerdá X, Boulay RR (2011a) Solar elevation triggers foraging activity in a thermophilic ant. Ethology 117:1031–1039

Amor F, Ortega P, Jowers MJ, Cerdá X, Billen J, Lenoir A, Boulay RR (2011b) The evolution of worker-queen polymorphism in *Cataglyphis* ants: interplay between individual and colony-level selections. Behav Ecol Sociobiol 65:1473–1482

Boulay R, Aron S, Cerdá X, Doums C, Hefetz A, Monnin T (2017) Social life in arid environments: the case study of *Cataglyphis* ants. Ann Rev Entomol 62:305–321

Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. Q Rev Biol 63:291–311

Cagniant H (1979) La parthénogénese thélytoque et arrhénotoque chez la fourmi *Cataglyphis cursor* Fonsc. (Hym. Form.). Cycle biologique en élevage des colonies avec reine et des colonies sans reine. Insect Soc 26:51–60

Cowan DP, Stahlhut JK (2004) Functionally reproductive diploid and haploid males in an inbreeding hymenopteran with complementary sex determination. Proc Natl Acad Sci USA 101:10374–10379

Cronin AL, Chifflet-Belle P, Fédéreci P, Doums C (2016) High intercolonial variation in worker nestmate relatedness and diverse social structure in a desert ant from Mongolia. Insect Soc 63:87–98

Crozier RH, Pamilo P (1996) Evolution of social insect colonies: sex allocation and kin selection. Oxford University Press, Oxford

Doums C, Cronin AL, Ruel C, Fédérici P, Haussy C, Tirard C, Monnin T (2013a) Facultative use of thelytokous parthenogenesis for queen production in the polyandrous ant *Cataglyphis cursor*. J Evol Biol 26:1431–1444

Doums C, Ruel C, Clémencet J, Fédérici P, Cournault L, Aron S (2013b) Fertile diploid males in the ant *Cataglyphis cursor*: a potential cost of thelytoky. Behav Ecol Sociobiol 67:1983–1993

Eyer PA, Leniaud L, Darras H, Aron S (2013) Hybridogenesis through thelytokous parthenogenesis in two *Cataglyphis* desert ants. Mol Ecol 22:947–955

Gobin B, Peeters C, Billen J (1998) Production of trophic eggs by virgin workers in the ponerine ant Gnamptogenys menadensis. Physiol Entomol 23:329–336

Greeff JM (1996) Effects of Thelytokous Worker Reproduction on Kin-Selection and Conflict in the Cape Honeybee, *Apis mellifera capensis*. Philisoph Trans R Soc B 351:134

Hammond RL, Keller L (2004) Conflict over male parentage in social insects. PLoS Biol 2:e248

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Berlin

Jowers MJ, Amor F, Ortega P, Lenoir A, Boulay R, Cerdá X, Galarza JA (2014) Recent speciation and secondary contact in endemic ants. Mol Ecol 23:2529–2542

Leniaud L, Darras H, Boulay R (2012) Social hybridogenesis in the clonal ant *Cataglyphis hispanica*. Curr Biol 22:1188–1193

Leniaud L, Hefetz A, Grumiau L, Aron S (2011) Multiple mating and supercoloniality in *Cataglyphis* desert ant. Biol J Linn Soc 104:866–876

Miyakawa MO, Mikheyev AS (2015a) Males are here to stay: fertilization enhances viable egg production by clonal queens of the little fire ant (Wasmannia auropunctata). Sci Nat 102:15

Miyakawa MO, Mikheyev AS (2015b) QTL mapping of sex determination loci supports an ancient pathway in ants and honey bees. PLoS Genet 11(11):e1005656

Pearcy M, Aron S, Doums C, Keller L (2004) Conditional use of sex and parthenogenesis for worker and queen production in ants. Science 306:1780–1783

Pearcy M, Goodisman MAD, Keller L (2011) Sib mating without inbreeding in the longhorn crazy ant. Proc R Soc Lond Ser B 278:2677–2681

Pearcy M, Timmermans I, Allard D, Aron S (2009) Multiple mating in the ant *Cataglyphis cursor*: testing sperm limitation and the diploid male load hypotheses. Insect Soc 56:94–102

R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rabeling C, Kronauer JC (2013) Thelytokous parthenogenesis in eusocial Hymenoptera. Annu Rev Entomol 58:273–292

Rispe C, Pierre JS, Simon JC, Gouyon PH (1998) Models of sexual and asexual coexistence in aphids based on constraints. J Evol Biol 11:685–701

Smith JM (1971) What use is sex? J Theor Biol 30:319–335

Teseo S, Kronauer DJC, Jaisson P, Châline N (2013) Enforcement of reproductive synchrony via policing in a clonal ant. Curr Biol 23:328–332

Timmermans I, Hefetz A, Fournier D, Aron S (2008) Population genetic structure, worker reproduction and thelytokous parthenogenesis in the desert ant *Cataglyphis sabulosa*. Heredity 101:490–498

Tsuji K (1995) Reproductive conflicts and levels of selection in the ant *Pristomyrmex pungens*: Contextual analysis and partitioning of covariance. Am Nat 146:586–607

West-Eberhard MJ (1981) Intragroup selection and the evolution of insect societies. In: Natural selection and social behavior. Alexander RD, Twinckle DW (eds), Chiron, New York, pp. 3–17

van Wilgenburg E, Driessen G, Beukeboom LW (2006) Single locus complementary sex determination in Hymenoptera: an “unintelligent” design? Front Zool 3:1