*Evolution of reproductive traits in* Cataglyphis desert ants: mating frequency, queen number, and thelytoky

## Serge Aron, Patrick Mardulyn & Laurianne Leniaud

#### **Behavioral Ecology and Sociobiology**

ISSN 0340-5443

Behav Ecol Sociobiol DOI 10.1007/s00265-016-2144-9





Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be selfarchived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".



ORIGINAL ARTICLE



# Evolution of reproductive traits in *Cataglyphis* desert ants: mating frequency, queen number, and thelytoky

Serge Aron<sup>1</sup> · Patrick Mardulyn<sup>1</sup> · Laurianne Leniaud<sup>1</sup>

Received: 12 December 2015 / Revised: 2 May 2016 / Accepted: 4 May 2016 © Springer-Verlag Berlin Heidelberg 2016

#### Abstract

Cataglyphis desert ants display unique variation in their breeding systems, making this genus a particularly interesting model to study the evolution of derived reproductive traits in eusocial Hymenoptera. Colonies may be headed by a single or several queens, and queens may be singly or multiply mated. Furthermore, in a number of species, both the workers and queens do reproduce asexually by thelytokous parthenogenesis. We examined whether the variability in reproductive traits is the result of a single evolutionary transition or of multiple independent evolutionary transitions, per trait. First, we inferred a phylogenetic estimate for the genus by analyzing DNA sequence variation among several species at four independent loci. Our phylogenetic hypothesis confirms the monophyly of previously defined species groups. Second, we examined the evolution of four reproductive traits in the genus (queen mating frequency, colony queen number, as well as worker reproduction and queen reproduction by thelytokous parthenogenesis), by inferring the state of these traits at the ancestral nodes. Our results show that polyandry and monogyny are the most likely conditions for the ancestor of the genus; the status of worker and queen thelytoky remains ambiguous. Genetic diversity within colonies may have been

Communicated by J. Heinze

**Electronic supplementary material** The online version of this article (doi:10.1007/s00265-016-2144-9) contains supplementary material, which is available to authorized users.

a major driver for the evolution of derived reproductive traits in *Cataglyphis*.

#### Significance statement

Kinship among group members has long been recognized as a main factor promoting the evolution of sociality and reproductive altruism. In eusocial Hymenoptera (ants, bees, wasps), at least three reproductive characteristics were shown to profoundly affect colony kin structure and may have played a key role in the evolution and maintenance of reproductive altruism: the queen mating frequency, the number of reproductive queens in a colony, and reproduction by parthenogenesis. We infer the evolution of these three reproductive traits in Cataglyphis desert ants, a genus displaying considerable variation in reproductive strategies. We propose a phylogenetic estimate for the genus, from variation at four loci. Then, we show that multiple evolutionary changes occurred for all three reproductive characters studied. Our results suggest that selection for enhanced colony genetic diversity has driven the evolution of reproductive traits away from their ancestral state.

**Keywords** Breeding system · Parthenogenesis · Phylogeny · Eusociality · Formicidae

#### Introduction

The Hymenoptera, where eusociality evolved 10 to 11 times independently (Hölldobler and Wilson 2009), have provided crucial empirical evidence for inclusive fitness theory (Hamilton 1964). The theory states that the fitness of workers can be enhanced indirectly by refraining from individual reproduction and increasing the reproductive output of their relatives. Comparative studies across a wide range of bees, wasps, and ants showed that close relatedness played a

Serge Aron saron@ulb.ac.be

<sup>&</sup>lt;sup>1</sup> Evolutionary Biology and Ecology, Université Libre de Bruxelles, 1050 Brussels, Belgium

decisive role in the evolution of this altruistic behavior. Both functional monogyny (a single reproductive queen per colony) and monandry (single mating by queens), two factors maximizing relatedness among colony members, were found to be the ancestral conditions in all lineages investigated (except a lineage of halictid bees where the ancestral state for queen number was uncertain), presumably setting up the stage for widespread adoption of eusocial behavior within the Hymenoptera (West et al. 2007; Hughes et al. 2008a, b; Boomsma 2009; Boomsma et al. 2009; Bourke 2011).

However, derived breeding systems affecting group genetic diversity and nestmate relatedness evolved secondarily in virtually all clades. In particular, multiple mating by queens (polyandry) and the occurrence of multiple reproductive queens in a colony (polygyny) are widespread (Crozier and Pamilo 1996; Hughes et al. 2008a, b; Boomsma et al. 2014). Polyandry and polygyny both decrease relatedness among workers and the brood they rear and, consequently, reduce the inclusive fitness benefits from helping.

Over the past decade, it has also become apparent that thelytokous parthenogenesis, the asexual production of female offspring by mated or unmated females, evolved repeatedly in distantly related groups. So far, it has been documented in 51 eusocial Hymenoptera (Rabeling and Kronauer 2013): the Cape honeybee Apis mellifera capensis and 50 species of ants. Thelytokous parthenogenesis has resulted in the evolution of an unexpectedly high diversity of reproductive strategies that directly affect the colony genetic architecture and relatedness among individuals (Wenseleers and Van Oystaeyen 2011). For example, queens of some species conditionally use sexual and asexual reproduction: workers arise from fertilized eggs, while new reproductive queens are produced by parthenogenesis and are clones of their mother (Pearcy et al. 2004, 2011; Fournier et al. 2005; Ohkawara et al. 2006). A few species evolved obligate parthenogenesis, with the consequence that colonies are entirely composed of clonal lines of genetically identical individuals (Tsuji 1988; Tsuji and Yamauchi 1995; Schilder et al. 1999; Rabeling et al. 2009; Kronauer et al. 2012).

*Cataglyphis* desert ants offer a unique opportunity to examine the evolution of derived reproductive traits in social insects. All species have very similar ecology; they inhabit arid lands and deserts, including the Sahara, Near East and Middle East, Arabian Peninsula, and Central Asia. Yet, the genus evolved an amazing diversity of breeding systems and modes of reproduction (Lenoir et al. 2009; Leniaud et al. 2011; Aron et al. 2013). Species-specific genetic analyses showed that queen mating frequency greatly varies, providing an almost tenfold variation in level of polyandry across species. There is also large interspecific variation in the number of queens per colony, whereas monogyny is the rule in some species, others are strictly polygynous. Furthermore, workers of all *Cataglyphis* species studied have kept ovaries, hence, the ability to lay unfertilized eggs. They can produce haploid (male) eggs by arrhenothokous parthenogenesis and, in some species, diploid (female) eggs by thelythokous parthenogenesis. Finally, queens of several species were shown to simultaneously harvest the benefits of clonality and sexuality, by producing new reproductive queens asexually through thelytokous parthenogenesis and workers by sexual reproduction.

We investigated whether the strong variability in reproductive traits displayed by the genus Cataglyphis is the result of a single evolutionary transition per trait or whether multiple independent evolutionary transitions occurred in each trait. To that end, we first inferred a phylogeny of the genus. To complement previous preliminary phylogenetic studies (Agosti 1990; Knaden et al. 2012), we collected DNA sequences from one mitochondrial and three nuclear genes on samples from 25 previously recognized species, including all species for which the reproduction system was previously documented. We then used the estimated phylogeny to reconstruct the evolution of reproductive traits in the genus. More specifically, we examined whether (i) single or multiple mating, (ii) monogyny or polygyny, (iii) worker reproduction by thelytokous parthenogenesis, and (iv) the capability of queens to produce new daughter queens by thelytokous parthenogenesis characterized the ancestor of the genus, and we assessed the number of evolutionary transitions that occurred for these traits, as well as their position on the estimated phylogeny.

#### Materials and methods

#### Sample collection

Our analysis includes 35 *Cataglyphis* samples, corresponding to at least 25 different species based on their morphological features (see ESM Online Resource 1). As outgroups, we used one species of *Proformica (Proformica nasuta)* and one of *Bajcaridris (B. sp.)*, two genera that are closely related to *Cataglyphis* (Blaimer et al. 2015), plus one distantly related species of *Formica (Formica wheeleri)*. Sequences for *P. nasuta* and *F. wheeleri* were obtained from GenBank (DQ353195, DQ353311, DQ353075, DQ352892, DQ353149, DQ353362, DQ353087, DQ352973). Our study does not include endangered or protected species; no specific authorizations were required for the capture, collection, and exportation of samples.

#### **DNA** sequence data

We extracted DNA from thorax and legs of 36 ants (35 *Cataglyphis* and one *Bajcaridris*) using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocols. For each specimen, three

nuclear genes (Wingless, Wg; abdominal-A, AB; longwave rhodopsin, LR) and one mitochondrial gene (COI) were amplified via PCR using published and designed primers (Online Resource 2). PCR amplifications were conducted in a volume of 25 µL, using TopTag DNA polymerase (Oiagen) and concentration of the various components of the PCR mix suggested by the manufacturer's protocol. Cycling conditions were as follows: After an initial denaturation step at 94 °C for 3 min, 35 cycles were performed of 1 min at 94 °C, 1 min at 47 °C (mitochondrial DNA) or 60 °C (nuclear DNA), and 2 min at 72 °C and followed by a final extension at 72 °C for 10 min. Cycle sequencing was performed using BigDye terminator mix v3.1 (Applied Biosystems), following the manufacturer's protocols and using the same primers used for the PCR amplification. Sequencing products were run on an automated ABI 3730 sequencer (Applied Biosystems, Foster City, USA). All DNA fragments were sequenced in both directions.

#### **Phylogenetic analyses**

Sequences were aligned with the Muscle algorithm (Edgar 2004) implemented in Aligner 3.7.1 (CodonCode Corporation, Dedham, MA, USA). JModeltest 0.1.1 (Huelsenbeck and Ronquist 2001) was used to choose the most appropriate nucleotide substitution model for each marker, according to the corrected Akaike information criterion (AICc) (Akaike 1974). Phylogenetic analyses were conducted in a Bayesian framework using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001), for each locus separately. A GTR + I + G model was selected for COI and a HKY + I model for the other loci, as these are the models closest to those suggested by JModeltest that were available in MrBayes. Two runs implementing four MCMC chains (three heated, one cold; heating parameter set to 0.2) of one to two million steps each were conducted. The cold chains were sampled every 500 steps, and burn-in was set to 25 % of samples. Results from the two independent runs were compared to ensure convergence towards the same topology. Convergence between runs was considered to be sufficient when the reported average standard deviation of split frequencies was below 0.001. To verify that the sampling was adequate when estimating the posterior probability distribution, we checked that no trend could be detected when plotting the log probability of the data versus generation of the run (ensuring stationarity). Other phylogenetic analyses were conducted on all loci, combined in a single data set, under the same conditions as above. Two different partition schemes were used in two separate MrBayes analyses: (1) data were divided in four partitions, corresponding to the four different loci, each partition being assigned a separate substitution model (topology and branch lengths being the same for all partitions), as described above; (2) data were partitioned according to the best partition identified by PartitionFinder version 1.1.1 (Lanfear et al. 2012). For this second case, a prior PartitionFinder run was launched, by defining a priori a partition for each codon position of each gene, plus a partition for the single LR intron (total of 13 partitions). The best partitioning identified by PartitionFinder divided the data set in seven partitions, each being assigned a separate substitution model: (1) COI codon position 1 (TrN + I + G), (2) COI codon position 2 (F81), (3) COI codon position 3 (TrN + I + G), (4) AB + Wg codon position 3 (HKY + I), (5) AB + Lr + Wg codon position 1, plus Wg codon position 2 (K80 + I), (6) AB codon position 2 (F81), and (7) Lr intron and codon positions 2 + 3 (K80 + G). For the MrBayes analysis, however, we replaced the suggested TrN and K80 models by the HKY model. Finally, a maximum likelihood analysis was conducted on the combined data set with PhyML version 3.1 (Guindon and Gascuel 2003), along with a bootstrap analysis. In this case, a HKY + I + G model was selected (no partitioning option available with that program).

Because our phylogeny differed from that proposed by Knaden et al. (2012) (see "Results"), we performed one additional analysis on the COI locus only, including all sequences available in GenBank for *Cataglyphis*. This aligned dataset included 114 sequences and was analyzed under the same conditions and with the same three outgroups, as before.

Output trees from all analyses were visualized with FigTree version 1.4.0 (http://tree.bio.ed.ac.uk/software).

#### **Evolution of reproductive traits**

Our study on the evolution of reproductive traits in *Cataglyphis* includes all 22 species for which data on reproduction have been reported to date. It concerns about 25 % of the species richness of the genus, which is estimated at 91 species (antcat.org). Our taxonomic sampling covers a wide diversity of breeding systems and modes of reproduction; sampled species are strictly monogynous or polygynous, mating frequency varies from single mating to obligate multiple mating, and some species reproduce by thelytokous parthenogenesis whereas others do not.

Using the phylogenetic estimate from the four loci combined (Bayesian tree of Fig. 1), we inferred the evolution of four reproductive traits: (i) queen mating system (single or multiple mating), (ii) colony queen number (monogyny or polygyny), (iii) worker reproduction by thelytokous parthenogenesis, as well as (iv) the capability of queens to produce new daughter queens by thelytokous parthenogenesis. We were able to assign accurately some or all of these traits to the 22 species based on the literature (Table 1).

In social Hymenoptera, the number of males a queen mates with is difficult to determine because mating typically occurs during large nuptial flights tens of meters above ground. Therefore, we determined the minimum number of mating Fig. 1 Phylogeny estimate inferred from four loci combined (Wg, LR, AB, and COI). The tree is a 50 % majority-rule consensus generated by MrBayes. Posterior probability values (*black*) and ML bootstrap values (*gray*) are reported along branches. *Vertical bars* to the right delimit species groups



per queen  $M_p$  on the basis of paternity frequency (Boomsma and Ratnieks 1996). Paternity frequency is defined as the number of males that father the offspring of a single queen; it is recognized as the more accurate estimate of the number of matings per queen in social Hymenoptera (Baer 2011; Jaffé et al. 2012). In our study, data on paternity frequencies are based on the number of patrilines found from motheroffspring genetic combinations and/or from direct genotyping of sperm stored in the spermatheca of the queen. Following Boomsma and Ratnieks (1996) classification, paternity frequency data allowed us to identify three classes of mating systems: obligatory multiple mating (paternity frequency always  $\geq 2$  and often  $\geq 4$  mating per queen), multiple-single mating (paternity frequency usually >1, with a minority of queens singly mated), and single-double mating (paternity frequency usually=1, with a minority of queens doubly mated) (see Table 1). No species was found obligatory single mated (strict monandry). Therefore, we coded each species as multiple (m), multiple-single (m-s), or single-double (s-d), respectively (see

Aron et al. 2015 for details on paternity frequency, proportion of queens being singly or multiply mated, and proportion of queens being mated with 2 to 14 males in different Cataglyphis species). Data on the colony queen number were obtained from field observations and from the genotype of workers from natural colonies. Polygyny was concluded only when genetic analyses confirmed that several queens contributed to offspring production. We scored species as monogynous (1 reproductive queen) or polygynous (>1 reproductive queen) if the latter occurs in >10 % of colonies examined (Hughes et al. 2008a). Data on worker and queen reproduction by thelytokous parthenogenesis found in the literature were obtained using laboratory assays and mother-daughter genetic comparisons, respectively. In queenless colonies, production of female offspring (workers or new queens) by workers was considered as evidence of worker thelytoky. In queenright colonies, production of sexual daughters harboring the same genotype of the colony queen at multiple loci was regarded as indication of queen thelytoky. The absence of reproduction by

#### Behav Ecol Sociobiol

Table 1	Paternity frequency v	values (the number	of males the	hat father the	offspring of	of a single	queen), color	y queen r	number, an	d thelytoky in
Cataglyph	is desert ants									

Species groups	Species	Paternity frequency					Queen number			Colony	Thelytoky	
		M <sub>p</sub>	Proportion of queens mated		Mating system	No. [range]	Proportion of colonies with		structure	Workers	Queens	
			1x	2x	>2x			1Q	>1Q			
cursor	C. (cursor) piliscapa	5.30 (23) <sup>a</sup>	0	4	96	m	1.05 [1–2] (61) <sup>b, c</sup>	92	8	М	yes <sup>t</sup>	Yes <sup>b</sup>
	C. cursor		0	10	100	m	1 (>100) <sup>s</sup>	100	0	М	Yes <sup>s</sup>	_
altisquamis	C. mauritanica	1.14 (21) <sup>d</sup>	86	14	0	s-d	6.94 [2–19] (16) <sup>d</sup>	0	100	Р	Yes <sup>d</sup>	Yes <sup>d</sup>
	C. velox*	2.37 (19) <sup>d</sup>	11	58	31	m-s	2.85 [2-9] (16) <sup>d</sup>	0	100	Р	Yes <sup>d</sup>	Yes <sup>d</sup>
							$1(30)^{z}$	100	0	М		
	C. altisquamis	2.41 (17) <sup>e</sup>	24	35	41	m-s	1 (30) <sup>e</sup>	100	0	М	_	Yes <sup>e</sup>
	C. hispanica*	1.07 (30) <sup>f</sup>	93	7	0	s-d	$1(38)^{f}$	100	0	М	Yes <sup>f</sup>	Yes <sup>f</sup>
							6.4 [1–14] (18) <sup>g</sup>	44	56			
bombycinus	C. sabulosa	3.14 (29) <sup>h</sup>	3	31	66	m-s	1 (29) <sup>h</sup>	100	0	М	Yes <sup>h</sup>	No <sup>h</sup>
	C. bombycina	5.70 (10) <sup>i</sup>	0	0	100	m	1 (17) <sup>i, j</sup>	100	0	М	No <sup>i</sup>	No <sup>i</sup>
emmae	C. emmae	3.05 (21) <sup>k</sup>	5	24	71	m-s	$1(33)^{k}$	100	0	М	_	No <sup>k</sup>
	C. tartessica	1.17 (6) <sup>1</sup>	83	17	0	s-d	$1(106)^{m}$	100	0	М	No <sup>1</sup>	No <sup>1</sup>
	C. floricola	1.33 (6) <sup>1</sup>	67	33	0	s-d	$1 (79)^n$	100	0	М	No <sup>1</sup>	No <sup>1</sup>
albicans	C. theryi	2.50 (12) <sup>i</sup>	0	58	42	m	$1(12)^{i}$	100	0	М	No <sup>i</sup>	No <sup>i</sup>
	C. livida	4.41 (17)°	0	18	82	m	1 (17) <sup>o</sup>	100	0	М	No <sup>o</sup>	No <sup>o</sup>
	C. iberica	_				-	1 (>80) <sup>u, v, w</sup>			М	No <sup>x</sup>	-
	C. cubica	_				-	1 <sup>x</sup> ()			М	No <sup>x</sup>	_
	C. ruber	_				-	1 <sup>x</sup> ()			М	No <sup>x</sup>	-
	C. albicans	_				-	1 <sup>x</sup> ()			М	No <sup>x</sup>	_
	C. rosenhaueri	_				_	1 (>30) <sup>v, w</sup>			М	No <sup>x</sup>	_
bicolor	C. savignyi	8.50 (8) <sup>p</sup>	0	0	100	m	1 (13) <sup>p</sup>	100	0	М	Yes <sup>p</sup>	No <sup>p</sup>
	C. niger	5.77 (23) <sup>p</sup>	0	3	87	m	5.8 <sup>\$</sup> [1–18] (9) <sup>p</sup>	0	100	Р	No <sup>p</sup>	No <sup>p</sup>
							n.a. [1–17] (7) <sup>q</sup>					
	C. viatica	5.50 (8) <sup>r</sup>	0	0	8	m	$1(8)^{r}$	100	0	М	Yes <sup>r</sup>	No <sup>r</sup>
	C. holgerseni	-					_			_	No <sup>y</sup>	_

Given are the absolute paternity (or mating) frequency ( $M_p$ ), the proportion of queens mated with one male, two males, or more than two males, the mating system classes (single-double, multiple-single, and obligatory multiple mating), the number of queens per colony *No*, the proportion of colonies with a single queen (1Q) or with more than a queen (>1Q), the structure monogynous or polygynous of colonies and asexual production of females by thelytokous parthenogenesis in workers and queens (i.e., asexual production of new daughter queens). The number in brackets is the sample size. Thelytoky: Worker reproduction occurs in queenless colonies, only. Thelytokous parthenogenesis was determined from laboratory observations (i.e., worker-produced females) and from queen–daughter genetic combinations

*m* obligatory multiple mated (paternity frequency always  $\geq 2$  and often  $\geq 4$  mating per queen), *m-s* multiple single mated (paternity frequency usually >1, with a variable minority of queens being singly mated), *s-d* single-double mated (paternity frequency usually  $\approx 1$ , with a minority of queens being doubly mated) (modified from Boomsma and Ratnieks 1996 and Aron et al. 2015), *M* monogynous species (>10 % of colonies headed by a single queen), *M<sub>p</sub>* absolute paternity (or mating) frequency is calculated as the arithmetic mean number of patrilines found from mother–offspring genetic combinations and/or from direct genotyping of sperm stored in the spermatheca of the queen, *No* mean number of queens per colony determined from field observations and/or from workers genotypes, – data not available

<sup>a</sup> Pearcy et al. (2009); <sup>b</sup> Pearcy et al. (2004); <sup>c</sup> Pearcy and Aron (2006); <sup>d</sup> Eyer et al. (2013b); <sup>e</sup> Kuhn (2013); <sup>f</sup> Leniaud et al. (2012); <sup>g</sup> Darras et al. (2014a); <sup>h</sup> Timmermans et al. (2008); <sup>i</sup> Leniaud et al. (2013); <sup>j</sup> Leniaud et al. (2015); <sup>k</sup> Jowers et al. (2013); <sup>1</sup> R. Boulay (unpublished data); <sup>m</sup> Amor and Ortega (2014); <sup>n</sup> Amor et al. (2011); <sup>o</sup> Timmermans et al. (2010); <sup>p</sup> Leniaud et al. (2011); <sup>q</sup> Nowbahari et al. (1994); <sup>r</sup> Aron et al. (2013); <sup>s</sup> Lenoir et al. (2018); <sup>u</sup> Cerda et al. (2002); <sup>v</sup> Plaza and Tinaut (1989); <sup>w</sup> Cerda (personal communication); <sup>x</sup> Cagniant (2009) and Cagniant (personal communication); <sup>y</sup> Aron (unpublished data); <sup>z</sup> Eyer and Boulay (unpublished data); \*Both monogynous and polygynous populations have been reported. In *C. hispanica*, only 1 out of 14 populations sampled was found polygynous and the species is considered monogynous; <sup>§</sup> *C. niger* forms large, polydomous colonies made of several nests maintaining exchanges of workers, brood, and food. The number of queens is given per nest from the same colony. The number of nests sampled (rather than the number of colonies) is given between brackets thelytokous parthenogenesis was concluded from experiments based on samples ranging from 20 to 79 new queens from 3 to 12 colonies according to species. Overall, this approach allowed us to gather reliable data on mating frequency, queen number, and thelytoky.

We inferred ancestral states for all four reproductive traits using Mesquite 3.01 (Maddison and Maddison 2015), with both a Markov k-state single parameter likelihood model and a parsimony criterion. While the likelihood model used assumes that all changes are equally probable, the use of a parsimony criterion allows the use of a step matrix to define a specific cost for each type of evolutionary transition. In the case of polyandry, the only trait with more than two character states, we also inferred ancestral states under maximum parsimony, by specifying a cost of 1 between s-d and m-s or m-s and m but a cost of 2 between s-d and m. With this coding scheme, we hypothesize that m-s is an intermediate evolutionary step between s-d and m, so that a direct transition from s-d to m, if possible, represents a stronger evolutionary change (and thus is less likely to occur than a transition from s-d to m-s or from m-s to m).

#### Results

#### Molecular phylogeny

New sequences are available from GenBank under accession numbers KX118445-KX118588. The AB, LR, Wg, and COI sequence alignments contained 606, 562, 416, and 628 nucleotides, respectively (including gaps). Of the analyzed 2212 bp, 460 sites were variable, and 278 sites were parsimony informative. Overall, the Bayesian phylogenetic tree estimates inferred for each locus separately (Online Resource 3) were mostly compatible with each other and with the estimates inferred from all loci combined (Fig. 1). The topologies of both Bayesian tree estimates inferred from all four loci combined, but with different data partition schemes, were identical, and most nodes were strongly supported, with Bayesian posterior probabilities >0.9 (see Fig. 1 for the estimate from the four loci partition scheme and Online Resource 4 for the estimate from the PartitionFinder partition scheme). A few conflicts did appear, always between the COI tree and one of the nuclear locus tree. For example, the *Cataglyphis niger-C*. sp. (1) clade supported by the LR locus is contradicted by the C. niger-C. savignyi (1) clade supported by COI; similarly, the clade grouping three C. savignvi sequences on the COI tree is incompatible with the topology supported by the AB locus. These conflicts mostly concerned alternative relationships inside one of the species Fig. 2 Evolution of four reproductive traits in *Cataglyphis*. Evolution of ► reproductive traits as estimated along the tree of Fig. 1 under maximum likelihood (Markov k-sate 1 parameter model). Proportional likelihoods are reported for ancestral nodes, when available. Because several species are not monophyletic (see Fig. 1), only populations for which data on reproductive traits are available are considered

groups. One conflict between the COI gene, on the one hand, and the LR and AB loci, on the other, regarded the evolutionary relationships among species groups (position of altisquamis group). However, the clade (altisquamis, (albicans, bicolor)) is supported by a posterior probability value of only 0.57 on the COI tree, while the alternative clade (altisquamis, emmae, bombycinus) supported by the LR and AB loci is associated with a higher posterior probability value (1 and 0.99, respectively) and is also found on the combined loci tree (posterior probability of 1). We verified that the conflicting topologies within species groups, supported by different loci, did not impact our interpretation of trait evolution discussed below (i.e., the Mesquite analyses were run on alternative topologies that included alternative clades that were strongly supported by at least one locus).

The estimated tree in Fig. 1 indicates that species delimitation in this genus still needs clarification, since several species are not monophyletic (C. emmae, C. velox, C. espaladeri, C. savignvi). This may indicate the existence of several cryptic species in the genus. On the other hand, the paraphyly/ polyphyly of different species is suggested mainly by the mitochondrial COI locus (the three nuclear loci contributing more to the resolution of phylogenetic relationships at a higher phylogenetic level, i.e., among species groups; see Online Resource 3). This pattern could therefore also have been created by the occurrence of hybridization among closely related species that led to mitochondrial genome introgressions. Indeed, an increasing number of studies report evidence for mitochondrial introgression in insects (e.g., Quinzin and Mardulyn 2014; Zakharov et al. 2009) including Cataglyphis ants (Darras and Aron 2015).

Finally, the maximum likelihood (ML) tree estimate (inferred from all loci combined, Online Resource 5) resulted in a tree similar to the Bayesian tree estimates, although some clades supported by low bootstrap values were different. These differences regarded relationships within the *albicans* species group and a clade grouping the two *C. emmae* on the ML tree. Bootstrap values of the ML analysis are reported on the tree of Fig. 1. We have verified that the alternative topology favored by the ML estimate did not modify the evolution of reproductive traits inferred with the Bayesian tree estimate (Fig. 2).

Our *Cataglyphis* phylogeny differs from that previously proposed by Knaden et al. (2012) about the position of the



species groups *cursor* and *altisquamis*. Based on a single COI locus, these authors suggested that the two groups form a clade. As shown in Fig. 1, our results contradict this hypothesis. To investigate the source of this contradiction, we performed a phylogenetic analysis on all COI samples of the genus *Cataglyphis* available in GenBank (i.e., including the samples of Knaden et al. 2012; see "Materials and methods"). Of the analyzed 628 bp, 282 sites were variable and 235 sites were parsimony informative. The results obtained (Online Resource 6) were highly consistent with those found when analyzing multiple loci (Fig. 1).

#### **Evolution of reproductive traits**

Maximum likelihood (Fig. 2) and parsimony (not shown) inferences of ancestral states generated similar results for all reproductive traits, except workers thelytoky for which the state of the ancestor to the genus differed. Under maximum parsimony, assuming distinct costs for different types of evolutionary transitions or considering all evolutionary changes to be of equal cost resulted in the exact same inference for polyandry.

Our analyses suggest that monogyny and obligate multiple mating were the ancestral states in *Cataglyphis* (proportional likelihoods of 0.85 and 0.92, respectively; Fig. 2). However, reduction in queen mating frequency evolved secondarily and independently at least twice: once in the *altisquamis* group (*C. mauritanica*, *C. velox*, *C. hispanica*, and *C. altisquamis*) and once in the *emmae* group (*C. floricola*, *C. tartessica*, *C. emmae*). Reversal was however not complete since no species was found to be exclusively singly mated (Table 1). As for colony queen number, polygyny evolved twice independently, in *C. niger* and in the *altisquamis* group, with *C. mauritanica* and *C. velox*. Within the *altisquamis* group, *C. hispanica* appears to have reversed back to monogyny.

Whether the ancestor of the genus was capable of worker and queen reproduction through thelytokous parthenogenesis remains uncertain (proportional likelihood close to 0.6 and 0.5, respectively; Fig. 2), although worker thelytoky was the most parsimonious state for the two oldest nodes on the phylogeny (not shown). We can infer from the tree that the switch (gain or loss) between the absence and presence of workers thelytoky occurred repeatedly, at least four times in the genus (most parsimonious hypothesis). This character occurs in species belonging to the phylogenetic groups cursor, altisquamis, bombycinus, and bicolor. Conversely, it seems completely absent in species of the albicans group. Using the same approach, the ability of mother queens to produce new reproductive daughters by thelytokous parthenogenesis was gained or lost at least twice during the evolution of the genus (i.e., two gains, two losses, or one loss and one gain).

#### Discussion

#### Phylogeny of Cataglyphis

We propose an updated molecular phylogenetic hypothesis of the desert ant genus Cataglyphis based on one mitochondrial (COI) and three nuclear (Wg, AB, LR) markers. It is consistent with the previous delimitation of species groups (Agosti 1990; Radchenko 2001) that appear indeed monophyletic (Fig. 1). However, the evolutionary relationships among species groups, in particular the position of the groups cursor and altisquamis, are different from those inferred in previous studies. While cursor and altisquamis have been considered sister groups until now, our phylogenetic estimate contradicts this hypothesis (Fig. 1 and Online Resource 4). Because most inferred phylogenetic relationships are supported with high probability and the present study is based on a more comprehensive data set, we consider our phylogenetic hypothesis to be more robust and used it to estimate the ancestral states of queen mating system, colony queen number, and reproduction by thelytokous parthenogenesis in the genus.

Our phylogenetic analysis, including all species for which data on reproduction have been reported to date, reveals multiple independent evolutionary transitions for the different reproductive traits studied. While data on reproduction is currently available for a portion of the genus only, investigating reproductive strategies (i.e., deciphering the colony genetic structure, the mating system, and use of thelytokous parthenogenesis by queens and workers) in additional *Cataglyphis* species could only reveal additional transitions in reproductive traits. Our conclusion of multiple independent transitions can therefore only be strengthened by the inclusion of additional species in the future. The evolution of each studied reproductive trait is discussed below.

#### Queen mating system

Our data provide two insights into the evolution of queen mating system in *Cataglyphis* ants.

First, they show that obligate multiple mating is the most likely condition for the ancestor of the genus *Cataglyphis* and has been maintained in most species. The sister genus of *Cataglyphis* is *Rossomyrmex* (Blaimer et al. 2015); in the single species studied so far (*Rossomyrmex minuchae*), half the queens are polyandrous and can mate with up to five males (Sanllorente et al. 2010). Multiple mating is even more frequent in *Proformica* (Fernandez-Escudero et al. 2002), the closest relative of both these genera. This suggests that polyandry was already occurring in the common ancestor of both genera. Obligate multiple mating is a derived trait in social Hymenoptera (Boomsma and Ratnieks 1996; Hughes et al. 2008a). It has been documented in a few taxa (reviewed in Boomsma et al. 2009), including *Vespula* wasps and *Apis* 

honeybees, as well as *Atta* and *Acromyrmex* leaf-cutting ants, *Pogonomyrmex* harvester ants, *Aenictus, Dorylus, Eciton*, and *Neivamyrmex* army ants, *Cardiocondyla, Plagiolepis* pygmy ants (Thurin et al. 2011), and *Cataglyphis* desert ants (this study). In leaf-cutting ants (Villesen et al. 2002) and army ants (Kronauer et al. 2007a), phylogenetic studies revealed that polyandry had a single evolutionary origin and was subsequently maintained in the descendent lineages of each respective clade. The present study indicates that a single origin to polyandry is also probable in the genus *Cataglyphis*.

Mating is assumed to be costly for females in terms of energy expenditure, exposure to predation, or sexually transmitted parasites and pathogens. It may also impose fitness costs because of sperm competition or metabolic costs of storing more sperm. To be adaptive, the fitness benefits of multiple mating must outweigh these cumulative costs (Daly 1978; Chapman et al. 2003). Several hypotheses have been proposed to account for the evolution of polyandry in eusocial Hymenoptera; they are based on the benefits of enhanced genetic diversity within the worker force, the reduction in fitness load associated with production of sterile diploid males, or the acquisition of a sufficient reserve of sperm by queens to maintain large and long-lived colonies (reviewed in Crozier and Fjerdingstad 2001; Strassmann 2001; Boomsma et al. 2009). In desert ants, three fitness benefits, all based on the advantages of increased genetic diversity within colonies, seem relevant for the evolution of multiple mating by queens. (1) Polyandry may allow a more efficient division of labor by increasing the production of genetically diverse workers varying in their inclination to perform different tasks (Robinson and Page 1988; Julian and Cahan 1999; Hughes et al. 2003; Jones et al. 2004; Mattila and Seeley 2007; Wiernasz et al. 2008). In line with this, empirical studies showed that task preference is at least partly genetically influenced in Cataglyphis (cursor) piliscapa, with workers from different patrilines differing in their propensity to perform a given task in the colony (Eyer et al. 2013a). (2) Polyandry may favor social harmony by reducing conflicts between the queen and the workers over the maternity of males (Trivers and Hare 1976; Ratnieks 1988; Ratnieks et al. 2006). Workers of most Cataglyphis ants are capable of producing haploid males from unfertilized eggs. However, multiple mating results in that workers are, on average, more closely related to brothers than to nephews (sons of other workers). They are therefore selected to rear the queens' sons instead of the workers' sons. In line with this, in all Cataglyphis species studied so far, worker reproduction was indeed documented in queenless colonies only (Cagniant 1980, 2009; Timmermans et al. 2008). Determining the level of worker reproduction as a function of queen mating frequency would be a more valuable test of the role of relatedness on conflict resolution over the maternity of males in Cataglyphis. (3) Polyandry can enhance colonylevel resistance to pathogens (Schmid-Hempel 1998; Sherman et al. 1998; Baer and Schmid-Hempel 1999; Tarpy 2003; Hughes and Boomsma 2004). To our knowledge, no empirical study has been performed to test this issue in *Cataglyphis*. Yet, it may be particularly significant for this group, in which all species are scavengers and workers are likely to be contaminated with various pathogens growing on dead arthropods (Cerda et al. 1989).

Second, our data show that a reduction in mating frequency appeared secondarily and independently at least two times in the genus. While most species belong to the obligate multiple mating class, reversion to multiple-single and single-double mating occurred in the species groups altisquamis and emmae. Reversal was however not complete since no species was found to be exclusively singly mated. This is consistent with studies in two other ant genera, showing that species descending from ancestors with obligate multiple mating do not return to complete single mating (Acromyrmex echinatior, Sumner et al. 2004; Neivamyrmex carolinensis, Kronauer and Boomsma 2007b). Several factors may account for the reduction in queen mating frequency found in Cataglyphis. (i) Reduction in mating frequency occurred at least once (and possibly twice) in the altisquamis group. Interestingly enough, this group has evolved a hybridogenetic mode of reproduction (Leniaud et al. 2012; Eyer et al. 2013b; Kuhn 2013; Darras et al. 2014b). Populations are characterized by the coexistence of two distinct genetic lineages. Queens are almost always found mated with a male of the alternative lineage. Workers are pure hybridogens; they develop solely from hybrid crosses between the lineages. In contrast, new queens are pure-lineage individuals produced asexually by thelytokous parthenogenesis or, rarely, sexually from intralineage mating. The loss of genetic diversity within colonies due to reduction in queen mating frequency might be partly compensated by the high level of somatic heterozygosity in workers and the associated benefits from heterosis effects (Bullini 1994; Leniaud et al. 2012). (ii) Mating frequency also decreased in the species group emmae, in C. floricola and C. tartessica that belong to the single-double mating category. Colonies of these species are made of a single queen (strict monogyny) and a few dozen workers; their colony size is the smallest reported among Cataglyphis ants (Amor et al. 2011; Amor and Ortega 2014). A single mating may possibly supply enough sperm for fertilizing all the female's batch of eggs. This is consistent with the "multiple mating for more sperm" hypothesis (Cole 1983), which predicts that mating frequency is positively selected with increasing colony size to ensure storage of a greater supply of sperm. While this explanation may partly justify the reversal in queen mating frequency in C. floricola and C. *tartessica*, it is however unlikely to account for the variation in paternity frequency across species found in the entire genus (Aron et al. 2015). In Cataglyphis, the size of mature colonies is relatively small, ranging from a few hundreds to a few thousands individuals. In addition, neither absolute

mating frequency ( $M_p$ ) nor mating system class (m, m-s, s-d; Table 1) is associated with mature colony size (phylogenetically controlled generalized least-squared regressions analyses; Freckleton et al. 2002:  $r^2 = 0.14$ , F = 2.13, p = 0.17, n = 13and  $r^2 = 0.23$ , F = 2.76, p = 0.13, n = 13, respectively).

#### **Colony queen number**

Our results also show that monogyny is the most likely condition of the ancestor of *Cataglyphis* and that polygyny evolved twice in the genus, once in the *altisquamis* group (*C. velox* and *C. mauritanica*; in *C. hispanica*, populations are typically monogynous but rare facultatively polygynous populations were also documented; Darras et al. 2014a) and once in the *bicolor* group (*C. niger*).

Evolution of polygyny suggests that the benefits procured by co-breeding females outweigh its costs in terms of sharing reproduction and the resulting erosion in relatedness within colonies (Keller 1993; Bourke and Heinze 1994). Polygyny may be selected for if increasing genetic diversity within colonies brings better resistance against pathogens (Hughes and Boomsma 2004) or enhances colony productivity and longevity (Nonacs 1988; Keller 1993; Dalecky et al. 2005). By increasing colony size, polygyny may also provide competitive advantage for access to temporal food sources (Boulay et al. 2014).

The most likely explanation for the origin of polygyny in hybridogenetic species of the *altisquamis* group is the adoption of related queens into established colonies, as these queens have a certain probability of being mated with males from the same lineage, hence, unable to produce workers and found new colonies. Indeed, previous studies in C. hispanica showed that all queens carry alternate lineage sperm in monogynous populations, whereas about 15 % of queens carry same lineage sperm in polygynous populations (Darras et al. 2014a). In hybridogenetic species, queens mated with males from the same lineage cannot produce worker offspring but sexual offspring only (see above). Therefore, they cannot found new colonies on their own. Their only option is to stay in the natal colony, where they can contribute to the production of sexual offspring. Consistent with re-adoption of daughter (clonal) queens, the relatedness among nestmate queens in polygynous colonies is close to 0.90 (Darras et al. 2014a). Similar results were obtained in multiple-queen colonies of C. mauritanica and C. velox (Eyer et al. 2013b), where relatedness among queens within colonies is 0.98 and 0.92, respectively.

Polygyny in *C. niger* may be attributed to several factors, among which constraints on dispersal abilities could play a major role (Saar et al. 2014). This species is restricted to small sandy patches scattered along urbanized areas. Because suitable patches are probably rare, young queens leaving a patch risk failing to locate another one, increasing dispersal costs.

Consistent with adoption of daughter queens, nestmate queens are positively related in *C. niger* (Leniaud et al. 2011). Furthermore, as is often the case in polygynous species, *C. niger* forms polydomous colonies each composed of several nest units maintaining exchanges of queens, workers, and brood (Leniaud et al. 2011; Saar et al. 2014). As occupation of patch increases, so do nest density and the degree of habitat saturation, making polygyny even more likely. By increasing colony size, polygyny (and polydomy) in *C. niger* may also provide ecological dominance. For example, large colonies may gain competitive advantage for access to and defense of temporal food sources, by recruiting faster and more workers. They may also enhance colony defense, homeostasis, work ability and productivity, or year-to-year survival (Keller 1993; Bourke 1999; Boulay et al. 2014).

#### Thelytokous parthenogenesis in workers and queens

In eusocial Hymenoptera, the ability to lay unfertilized, diploid eggs giving rise to female offspring by thelytokous parthenogenesis has been reported in a handful of ants and the honeybee (Rabeling and Kronauer 2013). Yet, worker reproduction by thelytokous parthenogenesis is widespread in the genus *Cataglyphis*. It has been documented in all species groups, except *emmae*, and is particularly frequent in the *cursor* and *altisquamis* groups (Fig. 2). While the parsimony criterion suggests workers thelytoky to be the ancestral state of the genus, the likelihood criterion generates a more ambiguous estimate. Nonetheless, whatever the state of the ancestor, our phylogenetic hypothesis requires that the appearance/ disappearance of this trait has occurred at least four times during the evolution of the genus, making it a particularly labile character.

It has been proposed that worker reproduction by thelytokous parthenogenesis was selected for to counter high queen mortality, allowing workers to replace the queen(s) when she/they die (Lenoir et al. 1988; Dartigues and Lenoir 1990). Following this hypothesis, one would predict worker reproduction by thelytoky to be more frequent in monogynous than polygynous species, since monogyny increases the probability that queenless conditions arise compared to polygyny. Recent genetic analyses support the assumption that queen replacement is indeed a common phenomenon in queenless colonies of C. (cursor) piliscapa (Pearcy et al. 2006; Cheron et al. 2011) and Cataglyphis sabulosa (Timmermans et al. 2008), two species in which colonies are headed by a single queen with relatively short lifespan. However, in contradiction to this hypothesis, worker thelytoky seems to have been lost in some lineages of the bicolor group and in all lineages of the albicans group, where colonies are also typically monogynous (except C. niger).

Our current knowledge on the ability of queens to produce sexual daughters (new queens) by thelytokous parthenogenesis in Cataglyphis is more scarce, as it has been studied in 15 species only. So far, queen thelytoky was detected in five species belonging to two separate clades, the cursor and the altisquamis species groups. The inference of the ancestral state of the genus is ambiguous, whether we use a parsimony or likelihood criterion (absence/presence of the trait equally possible), but our phylogenetic hypothesis requires at least two character changes during the evolution of the genus (Fig. 2). These transitions are highly reliable since they are based on large samples of mother-sexual daughter genetic comparisons. Asexual reproduction is predicted to confer a twofold advantage relative to sexual reproduction, since it allows females the transmission of twice the number of genes to offspring each generation (Maynard Smith 1978). However, this benefit may be offset by the lack of genetic recombination and reduced genetic diversity of offspring, which can be detrimental in changing environments or in arms races with co-evolving parasites. In Cataglyphis, the production of workers sexually and new queens asexually enables queens to experience the advantages of both modes of reproduction. Colonies benefit both from a genetically diverse workforce, while queens can benefit from transmitting their genetic material undiluted to the next generation of queens (Pearcy et al. 2004). The genus *Cataglyphis* is exclusively distributed in dry areas around the world. All species occupy similar arid ecosystems and are highly thermophilic. Why queen thelytoky evolved in two species groups only, cursor and altisquamis, remains enigmatic.

In conclusion, our phylogenetic hypothesis shows that multiple evolutionary changes have occurred during the history of the desert ant Cataglyphis. Both obligate multiple mating by queens and monogyny appear the most likely conditions for the ancestor of the genus. These traits have been maintained in a majority of living species; however, reduction in mating frequency appeared secondarily in at least two species groups and polygyny evolved at least twice in the genus. While single mating and monogyny ensures a higher relatedness among individuals within a colony, which is thought to have been essential for the evolution and maintenance of eusociality, these characters have evolved multiple times in Cataglyphis ants showing that other selection pressures also influence reproduction in the genus. Our results suggest that the need for enhanced genetic diversity within colonies may have played an important role in this respect. Whether worker and queen reproduction by thelytokous parthenogenesis is the ancestral state remains unfortunately ambiguous. However, our data support that worker thelytoky is particularly labile since its appearance/disappearance occurred repeatedly during the evolution of the Cataglyphis. Finally, it should be noted that the inference of reproductive traits for the ancestor of the genus Cataglyphis at least partially depends on the species sampled for the analysis. Because only a portion of all species were sampled, and reproductive traits are currently known for an even smaller portion of the genus, our inference of the traits for the ancestor of the genus could be modified in the future by inclusion of additional species. On the other hand, our conclusion of multiple independent transitions in these traits could only be strengthened.

**Acknowledgments** We are grateful to F. Amor R. Boulay, H. Cagniant, X. Cerdà, C. Galkowski, C. Lebas and A. Lenoir for samples and for sharing unpublished data. This work was supported by several grants from the Belgian Fonds National pour la Recherche Scientifique (LL, PM, SA) and the Université Libre de Bruxelles (ARC 2010-2015 #5) (SA).

#### Compliance with ethical standards

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of interest** The authors declare that they have no conflict of interest.

#### References

- Agosti D (1990) Review and reclassification of *Cataglyphis* (Hymenoptera Formicidae). J Nat Hist 24:1457–1506
- Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723
- 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, Jowers MJ, Cerda X, Billen J, Lenoir A, Boulay RR (2011) The evolution of worker-queen polymorphism in Cataglyphis ants: interplay between individual- and colony-level selections. Behav Ecol Sociobiol 65:1473–1482. doi:10.1007/ s00265-011-1157-7
- Aron S, Darras H, Eyer PA, Leniaud L, Pearcy M (2013) Structure génétique des sociétés et systèmes d'accouplement chez la fourmi *Cataglyphis viatica* (Fabricius 1787). Bull Inst Sci Rabat 35:103– 109
- Aron S, Lybaert P, Baudoux C, Vandervelden M, Fournier D (2015) Sperm production characteristics vary with level of sperm competition in *Cataglyphis* desert ants. Funct Ecol. doi:10.1111/1365-2435. 12533
- Baer B (2011) The copulation biology of ants (Hymenoptera: Formicidae). Myrmecol News 14:55–68
- Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151– 154
- Blaimer BB, Brady SG, Schultz TR, Lloyd MW, Fisher BL, Ward PS (2015) Phylogenomic methods outperform traditional multi-locus approaches in resolving deep evolutionary history: a case study of formicine ants. BMC Evol Biol 15:271
- Boomsma JJ (2009) Lifetime monogamy and the evolution of eusociality. Philos Trans R Soc B 364:3191–3207
- Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. Philos Trans R Soc B 351:947–975
- Boomsma JJ, Kronauer DJC, Pedersen JS (2009) The evolution of social insect mating systems. In: Fewell J, Wilson EO, Gadau J (eds) Organization of insect societies. Harvard University Press, Cambridge, pp 1–25
- Boomsma JJ, Huszar DB, Pedersen JS (2014) The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. Anim Behav 92:241–252

- Boulay R, Arnan X, Cerda X, Retana J (2014) The ecological benefits of larger colony size may promote polygyny in ants. J Evol Biol 27: 2856–2863. doi:10.1111/jeb.12515
- Bourke AFG (1999) Colony size, social complexity and reproductive conflict in social insects. J Evol Biol 12:245–257
- Bourke AFG (2011) Principles of social evolution. Oxford University Press, Oxford
- Bourke AGF, Heinze J (1994) The ecology of communal breeding: the case of multiple-queen leptothoracine ants. Philos Trans R Soc B 345:359–372
- Bullini L (1994) Origin and evolution of animal hybrid species. Trends Ecol Evol 9:422–426
- Cagniant H (1980) La parthénogenèse thélytoque et arrhénotoque chez la fourmi *Cataglyphis cursor* Fonsc. Etude en élevage de la productivité de sociétés avec reine et de sociétés sans reine. Insect Soc 27:157–174
- Cagniant H (2009) Le genre *Cataglyphis* Foerster, 1850 au Maroc (Hyménoptères Formicidae). ORSIS 24:41–71
- Cerda X, Retana J, Bosch J, Alsina A (1989) Daily foraging activity and food collection of the thermophilic ant *Cataglyphis cursor* (Hymenoptera Formicidae). Vie Milieu 39:207–212
- Cerda X, Dahbi A, Retana J (2002) Spatial patterns, temporal variability, and the role of multi-nest colonies in a monogynous Spanish desert ant. Ecol Entomol 27:7–15
- Chapman T, Arnqvist G, Bangham J, Rowe L (2003) Sexual conflict. Trends Ecol Evol 18:41–47
- Cheron B, Monnin T, Federici P, Doums C (2011) Variation in patriline reproductive success during queen production in orphaned colonies of the thelytokous ant *Cataglyphis cursor*. Mol Ecol 20:2011–2022. doi:10.1111/j.1365-294X.2011.05075.x
- Cole BJ (1983) Multiple mating and the evolution of social behavior in the Hymenoptera. Behav Ecol Sociobiol 12:191–201
- Crozier RH, Fjerdingstad EJ (2001) Polyandry in social Hymenoptera disunity in diversity? Ann Zool Fenn 38:267–285
- Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Sex allocation and kin selection. Oxford University Press, Oxford
- Dalecky A, Gaume L, Schatz B, Mckey D, Kjellberg F (2005) Facultative polygyny in the plant-ant *Petalomyrmec phylax* (Hymenoptera: Formicinae): sociogenetics and ecological determinants of queen number. Biol J Linn Soc 86:133–151

Daly M (1978) The cost of mating. Am Nat 112:771-774

- Darras H, Aron S (2015) Introgression of mitochondrial DNA among lineages in a hybridogenetic ant. Biol Lett 11:20140971
- Darras H, Kuhl A, Aron S (2014a) Genetic determination of female castes in a hybridogenetic desert ant. J Evol Biol 27:2265–2271. doi:10. 1111/jeb.12470
- Darras H, Leniaud L, Aron S (2014b) Large-scale distribution of hybridogenetic lineages in a Spanish desert ant. Proc R Soc London, Ser B 281:20132396
- Dartigues D, Lenoir A (1990) La ponte des ouvrières chez *Cataglyphis bicolor* F. (Hymenoptera: Formicidae): mise en évidence d'une parthénogenèse thélytoque. Ann Soc Entomol Fr 26:121–123
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. doi: 10.1093/nar/gkh340
- Eyer PA, Freyer J, Aron S (2013a) Genetic polyethism in the polyandrous desert ant *Cataglyphis cursor*. Behav Ecol 24:144–151
- Eyer PA, Leniaud L, Darras H, Aron S (2013b) Hybridogenesis through thelytokous parthenogenesis in two *Cataglyphis* desert ants. Mol Ecol 22:947–955. doi:10.1111/mec.12141
- Fernandez-Escudero I, Pamilo P, Seppä P (2002) Biased sperm use by polyandrous queens of the ant *Proformica longiseta*. Behav Ecol Sociobiol 51:207–213
- Fournier D, Estoup A, Orivel J, Foucaud J, Jourdan H, Le Breton J, Keller L (2005) Clonal reproduction by males and females in the little fire ant. Nature 435:1230–1234

- Freckleton RP, Harvey PH, Pagel M (2002) Phylogenetic analysis and comparative data: a test and review of evidence. Am Nat 160:712– 726. doi:10.1086/343873
- Guindon S, Gascuel O (2003) PhyML: "a simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood". Syst Biol 52:696–704
- Hamilton WD (1964) The genetical evolution of social behavior. J Theor Biol 7:1–52
- Hölldobler B, Wilson EO (2009) The superorganism: the beauty, elegance, and strangeness of insect societies. W.W. Norton & Company, New York
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:745–755
- Hughes WOH, Boomsma JJ (2004) Genetic diversity and disease resistance in leaf-cutting ant societies. Evolution 58:1251–1260
- Hughes WOH, Sumner S, Van Borm S, Boomsma JJ (2003) Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. Proc Natl Acad Sci-Bio 100:9394–9397
- Hughes WO, Oldroyd BP, Beekman M, Ratnieks FLW (2008a) Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science 320:1213–1216
- Hughes WOH, Ratnieks FLW, Oldroyd BP (2008b) Multiple paternity or multiple queens: two routes to greater intracolonial genetic diversity in the eusocial Hymenoptera. J Evol Biol 21:1090–1095
- Jaffé R, Garcia-Gonzalez F, den Boer SPA, Simmons LW, Baer B (2012) Patterns of paternity skew among polyandrous social insects: what can they tell us about the potential for sexual selection? Evolution 66:3778–3788. doi:10.1111/j.1558-5646.2012.01721.x
- Jones JC, Myerscough MR, Graham S, Oldroyd BP (2004) Honey bee nest thermoregulation: diversity promotes stability. Science 305: 402–404
- Jowers MJ, Leniaud L, Cerda X, Alasaad S, Caut S, Amor F, Aron S, Boulay R (2013) Social and population structure in the ant *Cataglyphis emmae*. PLoS One 8:e72941. doi:10.1371/journal. pone.0072941
- Julian GE, Cahan S (1999) Undertaking specialization in the desert leafcutter ant Acromyrmex versicolor. Anim Behav 58:437–442
- Keller L (1993) Queen number and sociality in insects. Oxford University Press, Oxford
- Knaden M, Tinaut A, Stokl J, Cerda X, Wehner R (2012) Molecular phylogeny of the desert ant genus *Cataglyphis* (Hymenoptera: Formicidae). Myrmecol News 16:123–132
- Kronauer DJC, Boomsma JJ (2007) Multiple queens means fewer mates. Curr Biol 17:R753–R755
- Kronauer DJC, Johnson RA, Boomsma JJ (2007) The evolution of multiple mating in army ants. Evolution 61:413–422
- Kronauer DJC, Pierce NE, Keller L (2012) Asexual reproduction in introduced and native populations of the ant *Cerapachys biroi*. Mol Ecol 21:5221–5235
- Kuhn A (2013) Hybridogenèse sociale chez les fourmis désertiques *Cataglyphis*. Dissertation, Université Libre de Bruxelles
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol 29:1695–1701
- Leniaud L, Heftez A, Grumiau L, Aron S (2011) Multiple mating and supercoloniality in *Cataglyphis* desert ants. Biol J Linn Soc 104: 866–876. doi:10.1111/j.1095-8312.2011.01772.x
- Leniaud L, Darras H, Boulay R, Aron S (2012) Social hybridogenesis in the clonal ant *Cataglyphis hispanica*. Curr Biol 22:1188–1193
- Leniaud L, Pearcy M, Aron S (2013) Sociogenetic organisation of two desert ants. Insect Soc 60:337–344. doi:10.1007/s00040-013-0298-2
- Leniaud L, Pearcy M, Taheri A, Aron S (2015) Testing the genetic determination of the soldier caste in the silver ant. Insect Soc 62:517–524. doi:10.1007/s00040-015-0431-5

- Lenoir A, Querard L, Pondicq N, Berton F (1988) Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Psyche 95:21–44
- Lenoir A, Aron S, Cerda X, Hefetz A (2009) *Cataglyphis* desert ants: a good model for evolutionary biology in Darwin's anniversary year—a review. In memoriam J. Kugler (1916–2007). Isr J Zool 39:1–32
- Maddison WP, Maddison DR (2015) Mesquite: a modular system for evolutionary analysis. Version 3.04
- Mattila HR, Seeley TD (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. Science 317:362–364
- Maynard Smith J (1978) The evolution of sex. Cambridge University Press, Cambridge
- Nonacs P (1988) Queen number in colonies of social Hymenoptera as a kin-selected adaptation. Evolution 42:566–580
- Nowbahari E, Soroker V, Hefetz A, Malherbe MC (1994) *Cataglyphis niger*, the first case of polygyny in the genus *Cataglyphis* (Hymenoptera: Formicidae). In: Lenoir A, Arnold G, Lepage M (eds), Les Insectes Sociaux (Proc. 12th Intern. Congr. IUSSI, Paris-Sorbonne). University Paris-Nord Press, Paris, pp 483
- Ohkawara K, Nakayama M, Satoh A, Trindl A, Heinze J (2006) Clonal reproduction and genetic caste differences in a queen-polymorphic ant, *Vollenhovia emeryi*. Biol Lett 2:359–363
- Pearcy M, Aron S (2006) Local resource competition and sex ratio in the ant *Cataglyphis cursor*. Behav Ecol 17:569–574
- 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, Hardy O, Aron S (2006) Thelytokous parthenogenesis and its consequences on inbreeding in an ant. Heredity 96:377–382
- Pearcy M, Timmermans I, Allard D, Aron S (2009) Multiple mating in the ant *Cataglyphis cursor*: testing the sperm limitation and the diploid male load hypothese. Insect Soc 56:94–102
- Pearcy M, Goodisman MAD, Keller L (2011) Sib mating without inbreeding in the longhorn crazy ant. Proc R Soc London, Ser B 278:2677–2681. doi:10.1098/rspb.2010.2562
- Plaza J, Tinaut A (1989) Descripcion de los hormigueros de *Cataglyphis* rosenhaueri (Emery, 1906) y *Cataglyphis iberica* (Emery, 1906) en diferentes biotopos de la provincia de Granada (Hym. Formicidae). B Asoc Espan Entomol 13:109–116
- Quinzin M, Mardulyn P (2014) Multi-locus DNA sequence variation in a complex of four leaf beetle species with parapatric distributions: mitochondrial and nuclear introgressions reveal recent hybridization. Mol Phylogenet Evol 78:14–24
- Rabeling C, Kronauer D (2013) Thelytokous parthenogenesis in eusocial Hymenoptera. Annu Rev Entomol 58:273–292
- Rabeling C, Lino-Neto J, Cappellari SC, Dos-Santos IA, Mueller UG, Bacci M (2009) Thelytokous parthenogenesis in the fungusgardening ant *Mycocepurus smithii* (Hymenoptera: Formicidae). Plos One 48:e6781. doi:10.1371/journal.pone.0006781
- Radchenko AG (2001) The phylogeny and faunogenesis of the genus *Cataglyphis* Foerster (Hymenoptera, Formicidae). Entomol Rev 81:951–958
- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am Nat 132:217–236
- Ratnieks FLW, Foster KR, Wenseleers T (2006) Conflict resolution in insect societies. Annu Rev Entomol 51:581–608

- Robinson GE, Page RE (1988) Genetic determination of guarding and undertaking in honey bee-colonies. Nature 333:356–358
- Saar M, Leniaud L, Aron S, Hefetz A (2014) At the brink of supercoloniality: genetic, behavioral, and chemical assessments of population structure of the desert ant *Cataglyphis niger*. Front Ecol Evol 2:1–10
- Sanllorente O, Hammond RL, Ruano F, Keller L, Tinaut A (2010) Extreme population differentiation in a vulnerable slavemaking ant with a fragmented distribution. Conserv Genet 11:1701–1710
- Schilder K, Heinze J, Gross R, Holldobler B (1999) Microsatellites reveal clonal structure of populations of the thelytokous ant *Platythyrea punctata* (Smith) (Hymenoptera; Formicidae). Mol Ecol 8:1497– 1507
- Schmid-Hempel P (1998) Parasites in social insects. Princeton Univ Press, Princeton
- Sherman PW, Seeley TD, Reeve HK (1998) Parasites, pathogens, and polyandry in social Hymenoptera. Am Nat 131:602–610
- Strassmann JE (2001) The rarity of multiple mating by females in the social Hymenoptera. Insect Soc 48:1–13
- Sumner S, Hughes WO, Pedersen JS, Boomsma JJ (2004) Ant parasite queens revert to mating singly. Nature 428:35–36
- Tarpy DR (2003) Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proc R Soc London, Ser B 270:99–103
- Thurin N, Sery N, Guimbretiere R, Aron S (2011) Colony kin structure and breeding system in the ant genus *Plagiolepis*. Mol Ecol 20: 3251–3260. doi:10.1111/j.1365-294X.2011.05161.x
- 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
- Timmermans I, Grumiau L, Hefetz A, Aron S (2010) Mating system and population structure in the desert ant *Cataglyphis livida*. Insect Soc 57:39–46. doi:10.1007/s00040-009-0048-7
- Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. Science 191:249–263
- Tsuji K (1988) Obligate parthenogenesis and reproductive division of labor in the Japanese queenless ant *Pristomyrmex pungens*. Comparison of intranidal and extranidal workers. Behav Ecol Sociobiol 23:247–255
- Tsuji K, Yamauchi K (1995) Production of females by parthenogenesis in the ant *Cerapachys biroi*. Insect Soc 42:333–336
- Villesen P, Murakami T, Schultz TR, Boomsma JJ (2002) Identifying the transition between single and multiple mating of queens in fungusgrowing ants. Proc R Soc London, Ser B 269:1541–1548
- Wenseleers T, Van Oystaeyen A (2011) Unusual modes of reproduction in social insects: shedding light on the evolutionary paradox of sex. BioEssays 33:927–937
- West SA, Griffin AS, Gardner A (2007) Evolutionary explanations for cooperation. Curr Biol 17:R661–R672. doi:10.1016/j.cub.2007.06. 004
- Wiernasz DC, Hines J, Parker DG, Cole BJ (2008) Mating for variety increases foraging activity in the harvester ant, *Pogonomyrmex* occidentalis. Mol Ecol 17:1137–1144
- Zakharov EV, Lobo NF, Nowak C, Hellmann JJ (2009) Introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiidae). Heredity 102:590–599