RESEARCH ARTICLE

# Genetic evidence for parthenogenesis in the small carpenter bee *Ceratina dallatoreana* (Apidae, Ceratinini) in its native distribution range

Michael Mikát[1,2,3], Jakub Straka[1]

**1** *Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic* **2** *Department of Biology, Faculty of Science, York University, Toronto, Canada* **3** *Department of General Zoology, Martin Luther University, Halle, Germany*

Corresponding author: Michael Mikát (Michael.mikat@gmail.com)

**Citation:** Mikát M, Straka J (2023) Genetic evidence for parthenogenesis in the small carpenter bee *Ceratina dallatoreana* (Apidae, Ceratinini) in its native distribution range. Journal of Hymenoptera Research 95: 199–213. https://doi.org/10.3897/jhr.95.87165

## Abstract

Arrhenotoky is the typical mode of reproduction in Hymenoptera. Diploid females develop from fertilized eggs, whereas haploid males originate from unfertilized eggs. However, some taxa of Hymenoptera have evolved thelytoky, in which diploid females originate parthenogenetically from unfertilized diploid eggs. In contrast to some other hymenopteran lineages, like ants and parasitic wasps, thelytoky is generally very rare in bees.

Here, we evaluated the frequency of thelytoky in the small carpenter bee *Ceratina dallatoreana*, which was previously assumed to be thelytokous. By comparing genotypes of microsatellite loci between mothers and their offspring, we found that all female offspring were genetically identical to their mothers. We conclude that parthenogenesis is the prevailing and perhaps obligate mode of reproduction in *C. dallatoreana*. We also classify the cytological mode of this parthenogenesis as apomixis, or automictic parthenogenesis with central fusion and extremely reduced or non-existing recombination, because offspring showed no decrease of heterozygosity.

Because sociality is influenced by relatedness and *Ceratina* are ancestrally facultatively social, the high relatedness afforded by parthenogenesis should associate with social living in the nest. In accordance with previous work, however, we found no social nests of *C. dallatoreana*.

## Keywords

Apidae, heterozygosity, relatedness, sociality, thelytoky, Xylocopinae

## Introduction

Sexual reproduction predominates in animals (Simon et al. 2003; Kooi et al. 2017). However, parthenogenesis (embryo development without fertilization) has evolved in many lineages (Normark 2003; Engelstädter 2008; Neiman and Schwander 2011; Thierry 2013; Gokhman and Kuznetsova 2018). Obligately parthenogenetic lineages are usually recent (Neiman et al. 2009; Fujita et al. 2020), and though obligate parthenogenesis can be successful in the short-term (microevolutionary scale), sexual reproduction is more successful in the long-term (macroevolutionary scale) (Neiman and Schwander 2011; Thierry 2013). The frequency of parthenogenesis varies across insect orders, but it is especially common in stick insects and mayflies (Tvedte et al. 2019; Liegeois et al. 2021).

Different types of parthenogenesis most likely evolved by different mechanisms, with each type showing characteristic modes of inheritance (Engelstädter 2008). Only females are present in obligately parthenogenetic populations. More commonly, however, parthenogenesis is facultative, co-occurring with sexual reproduction and both sexes present in the population (Normark 2003; Liegeois et al. 2021)

Cytological mechanisms of parthenogenesis influence the genetic diversity and heterozygosity in the population (Pearcy et al. 2006; Engelstädter 2017; Hörandl et al. 2020). The presence or absence of meiosis defines two general processes: 1) mitotic parthenogenesis in the absence of meiosis, and 2) meiotic parthenogenesis, in which meiosis is present but diploidy is restored by several different mechanisms (Stenberg and Saura 2009). If mitotic parthenogenesis persists in a population, heterozygosity will increase, because the meiotic recombination leading to the loss of alleles is absent (Schwander and Crespi 2009; Tsutsui et al. 2014; Tvedte et al. 2019). On the other hand, meiotic parthenogenesis should lead to decreased heterozygosity, because diploidy is restored by endomitosis or fusion of the products of meiosis. Therefore, the frequency of heterozygotes in offspring may be the same or smaller than that of a parent in each locus. Typically, automictic parthenogenesis with terminal fusion (fusion of sister pronuclei) leads to a rapid decrease in heterozygosity (Engelstädter 2017; Alavi et al. 2018). However, heterozygosity can be retained in the case of meiotic parthenogenesis with central fusion if crossing over is absent during meiosis (Stenberg and Saura 2009; Engelstädter 2017). In these cases, the different products of meiosis I merge, with complementary halves of the mother's genetic information. Therefore, the heterozygosity in populations with this type of meiotic parthenogenesis increases similarly as in populations with mitotic parthenogenesis, despite differences in the cytological mechanisms of parthenogenesis (Engelstädter 2017).

A haplodiploid sex determination system is widespread among Hymenoptera (Normark 2003; Kooi et al. 2017). Males originate from unfertilized eggs, and are therefore haploid. Females develop from fertilized eggs and are therefore diploid (Gerber and Klostermeyer 1970; Mueller 1991; Stubblefield and Seger 1994). The sex of offspring depends on whether the (mated) mother fertilizes an egg (Gerber and Klostermeyer 1970; Stubblefield and Seger 1994). Unmated females can produce only male offspring arrenotokously (Shukla et al. 2013).

Thelytokous reproduction has evolved repeatedly in Hymenoptera (Vorburger 2014; Kooi et al. 2017), such as sawflies and parasitic Hymenoptera (especially Chalcidoidea, Cynipoidea and Ichneumonoidea). It is found less frequently in aculeate Hymenoptera (Kooi et al. 2017). The best evidence for thelytoky in aculeate Hymenoptera is from social species (Wenseleers and Van Oystaeyen 2011; Goudie and Oldroyd 2018). It evolved repeatedly in ants and has been documented in at least 50 species to date (Heinze 2008; Rabeling and Kronauer 2013; Goudie and Oldroyd 2018). In bees, facultative thelytoky is known in *Apis mellifera capensis*, in which workers lay thelytokous eggs, usually not in the nest where they originated (Goudie and Oldroyd 2014, 2018). However, thelytoky is rare in solitary nesting and weakly social Hymenoptera, with few exceptions (Kooi et al. 2017). Based on the sex ratio in populations of *Ceratina* bees (Apidae: Xylocopinae), thelytoky is predicted to occur in several species, including *C. dallatoreana* (Daly 1966; Snelling 2003). Males are extremely rare in *C. dallatoreana* (Daly 1966, 1983), leading to the hypothesis that this species reproduces by thelytokous parthenogenesis. Here, we test this hypothesis.

*C. dallatoreana* females nest in broken dead stems with pith, constructing a linear series of cells (Daly 1966). Although facultative sociality is common in this genus (Sakagami and Maeta 1977; Rehan et al. 2009; Groom and Rehan 2018), social nests have not been detected in this species to date (Daly 1966; Mikát et al. 2022). This species is endemic to the Mediterranean region and Central Asia (Terzo 1998; Terzo and Rasmont 2004; Fig. 1), and has been introduced into California, USA (Daly 1966). We used microsatellite genetic markers to examine the frequency of parthenogenesis in different populations across the native range of *C. dallatoreana* and to assess if the allele frequency aligns with Hardy-Weinberg equilibrium. Moreover, we attempt to infer the mode of parthenogenesis from the pattern heterozygosity inheritance.



**Figure 1.** Western Palearctic Region, showing the native range of *Ceratina dallatoreana*, based on Terzo and Rasmont (2004, 2011) and new localities from this study. Red – range of *C. dallatoreana*. Black triangles – sources of samples for this study.

## Methods

We collected nests of *C. dallatoreana* in several locations across its native area of distribution, in Cyprus (2018, 2019), Italy (Puglia and Lazio regions, 2013 and 2017), Greece (Crete 2018, 2020), Albania (2018) and Tajikistan (2019) (Fig. 1). Coordinates of collection locations are shown in Suppl. material 1. Additionally, we analyzed females collected in Georgia (2013–2014) and North Macedonia (2014). We collected these females outside of their nests with nets or pan traps.

Nests were collected from natural nesting opportunities, as well as in stems broken or cut by human management. The most common nesting substrates were *Rubus* spp. and *Foeniculum vulgare*. In Cyprus and Crete we cut stems of these plants to increase nest density for ease of sampling several months later. To ensure that all inhabitants were inside the nest, nests were collected in the evening after 18:00 local time. Nests were opened lengthwise with garden clippers, and the number of adults, number of juveniles, and the stages of juveniles were noted. All individuals were preserved in 96% ethanol for further analysis.

### Extraction of DNA

We isolated DNA using the Chelex protocol (Coombs et al. 1999). DNA was usually isolated from part of an individual (one or two legs from adults or pupae, part of the body from most larvae), but whole eggs and whole bodies of small larvae were also used. Samples were transferred to microcentrifuge tubes and dried for at least three hours. Later, we added 8 μl of proteinase K and 50 μl of 10% Chelex solution. This mixture was vortexed and inserted into a thermo cycler. The mixture was heated to 55 °C for 50 min and 97 °C for 8 min then cooled. The mixture was then vortexed and inserted into a centrifuge. After this 30 μl of supernatant were transferred to a well in the PCR plate.

### Optimization of multiplex

We selected 12 female *C. dallatoreana* (9 from Cyprus and 3 from Tajikistan) for testing of microsatellite loci. We used microsatellite primers developed for *C. nigrolabiata* (Mikát et al. 2019). Fourteen microsatellite loci were arranged in two multiplexes. The first multiplex was previously applied to *C. nigrolabiata*, *C. chalybea* and *C. cyanea* (Mikát et al. 2019). The second multiplex contained six loci. Four were different from loci in the first multiplex (17 and 36 marked by 6FAM, 9 marked by VIC and 7 marked by PET), and two loci (12 and 51) were shared with the first multiplex but marked by a different color.

We evaluated results of amplification and obtained four possibilities for each locus: a) a locus was successfully amplified in all cases and was polymorphic (eight loci). b) a locus was successfully amplified in some cases and was polymorphic (two loci), c) a locus was amplified in all cases but was not polymorphic (three loci), or d) amplification of locus failed (one locus, Suppl. material 2: table S1).

Polymorphic and reliable loci were loci numbers 30, 23, 8, 67, 17, 36, 9, and 12 (Suppl. material 2: table S1), locus numbers corresponding to *C. nigrolabiata* (Mikát et al. 2019). However, we excluded locus 30 for overlap with same color-marked loci and locus 8 for interaction of primers with primers for another locus. Six microsatellite loci were thus retained for final analysis (Suppl. material 2: tables S1, S2).

## PCR and Fragmentation analysis

We used Type-it Multiplex PCR Master Mix (Quiagen) according to the manufacturer's protocol. Primers of six microsatellite loci were use in a concentration of 0.05 µmol/l. We used these PCR conditions: 95 °C for 15 min; 30 cycles of 94 °C for 30 sec, 60 °C for 90 sec, 72 °C for 60 sec; and finally 60 °C for 30 min. After PCR, we mixed 0.8 µl of PCR product with 8.8 µl of formamide and 0.4 µl of marker Liz 500 Size scanner (Applied Biosystems). We heated the mixture to 95 °C for 5 min and then cooled it to 12 °C. Fragmentation analysis was performed on a 16-capillary sequencer at the Laboratory of DNA Sequencing at the Biological section of Faculty of Science, Charles University, Prague. Identification of alleles was performed in Gene Marker (Soft Genetics) software.

## Analysis of ploidy and heterozygosity

We included mothers from nests and additional individuals in this analysis. We did not include offspring, as they had the same genotypes as their mothers. For each locus, we checked if an individual had one allele (homozygote) or two alleles (heterozygote). Individuals were considered diploid when heterozygous at least one locus. Individuals with only one allele at each locus were considered haploid. We analyzed 132 females (30 from Crete, 64 from Cyprus, 11 from Georgia, 12 from Italy, 9 from Tajikistan, 3 from Albania, and 3 from North Macedonia). We also analyzed one gynandromorph (individual with female head morphology and male abdomen morphology) from Tajikistan.

## Analysis of diversity of multilocus genotypes

We counted multilocus genotypes for different localities. As one locality we defined an area where collected samples are at most ten kilometers from each other. In this analysis, we included adult females. We present only data from localities where at least three adult females were genotyped.

## Analysis of deficit or surplus of heterozygotes

We used adult females for this analysis. We samples from two populations: Lefkara village, Cyprus (n = 50), and Georgioupoli village, Crete (n = 26). All individuals were collected at most 10 kilometers from each other in the same population. We calculated observed and expected heterozygosity using software Genepop, version 4.7.5. (Rousset 2020). Finally, we tested the possible deviation from Hardy-Weinberg equilibrium and

heterozygote excess (proportion of heterozygotes higher than in populations in Hardy-Weinberg equilibrium) or heterozygote deficiency (proportion of heterozygotes lower than in population in Hardy-Weinberg equilibrium), also using Genopop.

## Analysis of parthenogenesis

We compared the genotype of each mother with offspring from the same nest. We analyzed 188 offspring from 59 nests in total. For this analysis, we selected nests in which the mother and immature brood were present – nests in stages active brood nests or full brood nest. Nests in the active brood stage are nests where the mother currently perform provisioning of brood cells. These nests contained currently provisioned brood cells and in outermost brood cell was egg or this brood cell was only partially provisioned (Rehan and Richards 2010; Mikát et al. 2021). In contrast, full brood nests contained larvae or pupae in the innermost and outermost cells, as the females had already completed provisioning and were guarding their offspring until adulthood (Rehan and Richards 2010; Mikát et al. 2021). Sampled nests for this analysis were from the following locations: Albania (10 offspring, two nests); Crete (23 offspring, six nests); Cyprus (89 offspring, 32 nests), Italy (38 offspring; 10 nests) and Tajikistan (28 offspring, nine nests).

## Results

### Sex ratio of adults

We collected *C. dallatoreana* samples across its native range. In total, we found 476 adult females, one gynandromorph and no adult males. Of the adult females, we collected 253 in Cyprus, 137 in Crete, three in Albania, three in North Macedonia, 36 in Italy, 30 in Georgia, and 14 in Tajikistan. We found the gynandromorph in Tajikistan.

### Ploidy

All analyzed adult females from the maternal generation (n = 132) were heterozygotes in at least one locus. One female was a heterozygote in only one locus, while all others (n = 131) were heterozygotes in at least two loci. Thus, we determined that *C. dallatoreana* females are diploid. The gynandromorph was homozygous in all loci, therefore we considered this individual to be haploid.

### Heterozygosity

We generally detected high heterozygosity in our studied loci. Average heterozygosity across all locations and loci was 56.25%. However, heterozygosity differs between loci, with the highest proportion of heterozygotes at locus 36 (97.06%), and lowest proportion at locus 12 (4.41%). The proportion of heterozygotes in each locus across different geographical areas is shown in Table 1.

Allele frequency deviated from Hardy-Weinberg equilibrium for all loci in Georgioupoli (Crete) and in three of five variable loci in Lefkara (Cyprus). Heterozygosity was increased in some loci but decreased in others. Observed heterozygosity was significantly higher than expected for loci 36 and 9 in Georgioupoli (Crete) and 36 and 67 in Lefkara (Cyprus), but significantly lower for loci 17, 23 and 12 in Georgioupoli (Crete) and 17 in Lefkara (Cyprus) (Table 2). When we performed this analysis in reduced sample (n=26 for Lefkara, Cyprus, n=13 for Georgioupoli, Crete), which excludes possible close relatives sampled in the same shrub, we obtained the same pattern for Lefkara population and a very similar pattern for the Georgioupoli population (Suppl. material 2: table S4).

**Table 1.** Proportion of heterozygotes at each studied locus by geographical area. The category other includes samples from Albania (n = 3) and North Macedonia (n = 3).

| | | | | Proportion of heterozygotes in locus | | | | |
|---|---|---|---|---|---|---|---|---|
| Country | N | 17 | 36 | 23 | 9 | 12 | 67 | mean |
| Crete | 30 | 0.33 | 1.00 | 0.73 | 1.00 | 0.03 | 0.97 | 0.68 |
| Cyprus | 64 | 0.16 | 0.98 | 0.16 | 0.58 | 0.00 | 0.97 | 0.47 |
| Georgia | 11 | 0.73 | 0.91 | 0.64 | 0.72 | 0.18 | 0.82 | 0.67 |
| Italy | 12 | 0.58 | 0.83 | 0.25 | 0.75 | 0.00 | 0.50 | 0.49 |
| Tajikistan | 9 | 0.33 | 1.00 | 1.00 | 0.78 | 0.00 | 1.00 | 0.69 |
| Other | 6 | 0.50 | 1.00 | 0.33 | 1.00 | 0.00 | 0.67 | 0.58 |
| Total | 132 | 0.32 | 0.97 | 0.41 | 0.73 | 0.04 | 0.90 | 0.56 |

**Table 2.** Comparison of expected (HetEXP) and observed (HetOBS) proportions of heterozygotes. P-values of statistical tests from expected frequencies are shown: p(excess) = p-value of heterozygote excess test, p(deficit) = p-value of heterozygote deficit test, p(HW) = p-value test of difference from Hardy-Weinberg equilibrium in allele frequency. All calculation performed in software Genepop. Bold indicates significant values. Locus 12 in Cyprus population had only one allele, therefore excess or deficit of heterozygotes could not be calculated.

| Lefkara (Cyprus), N=50 | | | | | | |
|---|---|---|---|---|---|---|
| Locus | p(deficit) | p(excess) | p(HW) | HetEXP | HetOBS | n alelles |
| 17 | **0.0000** | 1.0000 | **0.0000** | 0.59 | 0.18 | 5 |
| 36 | 1.0000 | **0.0000** | **0.0000** | 0.54 | 0.98 | 4 |
| 23 | 1.0000 | 0.7339 | 1.0000 | 0.15 | 0.16 | 3 |
| 9 | 0.4326 | 0.5688 | 0.0900 | 0.63 | 0.58 | 3 |
| 12 | NA | NA | NA | 0.00 | 0.00 | 1 |
| 67 | 1.0000 | **0.0000** | **0.0000** | 0.64 | 0.96 | 3 |
| Georgioupoli (Crete), N=26 | | | | | | |
| Locus | p(deficit) | p(excess) | p(HW) | HetEXP | HetOBS | n alelles |
| 17 | **0.0027** | 0.9974 | **0.0000** | 0.61 | 0.27 | 6 |
| 36 | 1.0000 | **0.0048** | **0.0000** | 0.82 | 1.00 | 9 |
| 23 | **0.0176** | 0.9824 | **0.0000** | 0.70 | 0.69 | 4 |
| 9 | 1.0000 | **0.0000** | **0.0000** | 0.63 | 1.00 | 4 |
| 12 | **0.0007** | 1.0000 | **0.0003** | 0.15 | 0.00 | 3 |
| 67 | 0.3422 | 0.6578 | **0.0000** | 0.72 | 0.96 | 6 |

## Diversity of genotypes

We found a high diversity of multilocus genotypes. In all localities, we collected at least two multilocus genotypes. In Cyprus, we collected the largest sample in Lefkara (n = 50). In this location, we collected females with 26 multilocus genotypes. Eighteen of these genotypes were collected only once. Two of the most common genotypes had a frequency of 14.0% (7/50). We sampled another three locations in Cyprus: Agios Theodoros (n = 4, three multilocus genotypes); Mathiatis (n = 3, three multilocus genotypes); and Pyrgos (n = 5, four genotypes).

In Crete, we collected the largest sample in Georgioupoli (n = 26). In this location, we found 15 multilocus genotypes. The most common genotype had frequency 36.4% (9/26). Another sampled location was Chania airport, where we found three multilocus genotypes in three sampled individuals.

In Italy, we sampled at Pescariello (n = 5, five genotypes), Cassino (n = 3, two genotypes) and Santa Marinella (n = 3, three genotypes). In Georgia, we analyzed females from Vashlovani (n = 4, three genotypes) and Kvareli (n = 3, three genotypes). In Tajikistan, we analyzed samples from Shariston (n = 4, two genotypes).

## Comparison of maternal and offspring genotypes

Almost all offspring genotypes were identical to those of their mother (97.87%, 184/188 out of 59 nests). The same genotype in mother and offspring was found in all nests in Albania (two nests, 10 offspring), Crete (six nests, 23 offspring), and Italy (10 nests, 38 offspring). In Cyprus, we found one out of 89 offspring (32 nests) with a different genotype than its mother and in Tajikistan we found 3 such offspring out of 28 offspring (9 nests). However, all offspring for which we detected different genotypes from those of the mother had much lower detection peak for multiple microsatellite loci than most of the analyzed individuals. All four individuals contained at least one allele which was not shared with their mother. Two individuals from Tajikistan had both alleles different from the mother at least one locus. One individual from Cyprus and two from Tajikistan had a unique allele not found in any other individual. We can also exclude the effect of null alleles, because all four individuals were heterozygotes at least one locus with alleles that would disagree with the maternal genotype. Therefore, the apparent differences between offspring and maternal genotypes was the result of genotyping errors.

## Discussion

Previously, *Ceratina dallatoreana* was believed to reproduce parthenogenetically (Daly 1966, 1983), but without direct genetic evidence. We observed parthenogenesis in several locations in the Mediterranean (Albania, Italy, Crete, Cyprus) and central Asia (Tajikistan), providing evidence for parthenogenesis from a large part of the native

range of the species. As males are extremely rare in North Africa (Daly 1983) and California, where the species was introduced (Daly 1966), parthenogenesis would appear to be the prevailing or only mode of reproduction across populations.

Thelytokous parthenogenesis is rare in bees. Outside of *Apis mellifera capensis* (Rabeling and Kronauer 2013; Goudie and Oldroyd 2014), it was previously in evidence only in the genus *Ceratina*, where populations of several species were found without known males. These evidence include *C. acantha* (Slobodchikoff and Daly 1971), *C. dentipes* (Snelling 2003; Shell and Rehan 2019; M. Mikát unpublished data), *C. parvula* (Terzo et al. 2007; M. Mikát, unpublished data) and *C. dallatoreana* (Daly 1966). These species are not closely related, belonging to different subgenera (Rehan and Schwarz 2015; Ascher and Pickering 2020). Parthenogenesis is probably not the prevailing mode of reproduction in *C. dentiventris* and *C. sakagamii* which are considered to be the most closely related to *C. dallatoreana*, because they do not show a skewed sex ratio (Daly 1983; Terzo 1998). However males have not been found in *C. rasmonti*, which is known from only a few individuals and is closely related to *C. dallatoreana* (Terzo 1998). Given the distribution of parthenogenesis across *Ceratina* lineages, there may be a trend for parthenogenesis to arise in the *Ceratina* genus. Future research that includes the sampling of more species with a high-resolution phylogeny is needed for understanding evolution of parthenogenesis in this genus.

Although we found several offspring with genotypes that were not identical to genotypes of mothers, we suspect that these cases were the result of genotyping errors such as allelic dropout or false alleles. Situations in which offspring showed different genotypes from the mother were usually not compatible with scenarios of sexual reproduction. These results were also incompatible with any mode of parthenogenesis, because we detected alleles in offspring that were not detected in the mother. In two of four cases, offspring bear in at least one locus both alleles different from the mother. In case of parthenogenesis we can suppose allele loss, but not the rise of novel alleles.

Offspring resulting from parthenogenesis should bear only alleles shared by their mother. However, the cytology of parthenogenesis determines the rate of loss of heterozygosity from mother to offspring (Pearcy et al. 2006). We did not observe any heterozygosity loss, as all offspring were genetically identical to their mothers (when four improperly genotyped individuals are excluded) and are therefore clones of their mothers. This is compatible with two types of parthenogenesis: apomixis and automixis with central fusion. Apomixis is the more likely of the two, because under automixis with central fusion there would be at least some loss of heterozygosity due to recombination (Goudie and Oldroyd 2014; Engelstädter 2017). Empirical studies on organisms with central fusion automixis using microsatellites showed at least some heterozygosity loss (Rey et al. 2011; Fougeyrollas et al. 2015). Studies of *Apis mellifera capensis* show that homozygotes arise due to recombination, but they often die during early development. Therefore, high heterozygosity would be preserved by selection (Goudie and Oldroyd 2014). As we did not find any case of a homozygous offspring with a heterozygous mother even at the developmental egg stage, apomixis is the more probable mechanism.

We have shown that thelytokous parthenogenesis is the prevailing mode of reproduction in *C. dallatoreana*. However, there remains the question of whether sexual reproduction is only extremely rare or not occur at all. The existence of males is rarely reported for this species, but most of the reports of males could have been confused with closely related species (Daly 1983; Terzo 1998). Males are undoubtedly reported from California, where *C. dallatoreana* is invasive and no similar species are present (Daly 1966). However, the existence of males alone does not prove their involvement in reproduction. Strictly apomictic species usually have only one or a few genotypes in one location or region (Lorenzo-Carballa and Cordero-Rivera 2009; Ryskov et al. 2017). Although we detected some genotypes repeatedly, there was usually a high genotype diversity in each location, suggesting that sexual reproduction sometimes occurs in *C. dallatoreana*, even if rare.

The best documented examples of thelytoky in aculeate Hymenoptera are found among advanced eusocial species, and features of thelytoky are influenced by their social organization (Goudie and Oldroyd 2018). On the other hand, *Ceratina* are often facultatively social (Groom and Rehan 2018; Rehan 2020). Although most studied species are able to establish social colonies, the larger proportion of the population is solitary, and social colonies contain only two or a few females (Sakagami and Maeta 1977; Rehan et al. 2009; Groom and Rehan 2018; Mikát et al. 2022). Reversion to strict solitary nesting is also evident in some species (Groom and Rehan 2018; Mikát et al. 2020). Social nests have not yet been documented in *C. dallatoreana* (Daly 1966; Mikát et al. 2022), although the number of nests so far analyzed does not preclude the possibility of occasional sociality. This is quite surprising, because parthenogenesis may be expected to facilitate group living due to high relatedness between mother and offspring (Hamilton 1964). Moreover, two other species of *Ceratina* where parthenogenesis probably occurs (*C. dentipes* and *C. parvula*) are facultatively social (Terzo et al. 2007; Rehan et al. 2009; Mikát et al. 2022). The social status of one parthenogenetic species, *C. acantha* (Slobodchikoff and Daly 1971), is not yet known. *Ceratina* bees are an excellent group for the study of social evolution, due to their within- and between-species variability in social behavior (Groom and Rehan 2018; Rehan 2020). The existence of parthenogenesis in *Ceratina* species that are not closely related provides us unique system for study how between-species variability in relatedness influences social evolution.

## Acknowledgements

# References

Alavi Y, van Rooyen A, Elgar MA, Jones TM, Weeks AR (2018) Novel microsatellite markers suggest the mechanism of parthenogenesis in *Extatosoma tiaratum* is automixis with terminal fusion. Insect Science 25(1): 24–32. https://doi.org/10.1111/1744-7917.12373

Ascher JS, Pickering J (2020) Discover Life bee Species Guide and World Checklist (Hymenoptera: Apoidea: Anthophila). http://www.discoverlife.org/mp/20q?guide=Apoidea_species

Coombs NJ, Gough AC, Primrose JN (1999) Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. Nucleic Acids Research 27(16): e12-i–e12-iii. https://doi.org/10.1093/nar/27.16.e12-i

Daly HV (1966) Biological studies on *Ceratina dallatorreana*, an alien bee in California which reproduces by parthenogenesis (Hymenoptera: Apoidea). Annals of the Entomological Society of America 59(6): 1138–1154. https://doi.org/10.1093/aesa/59.6.1138

Daly HV (1983) Taxonomy and ecology of Ceratinini of North Africa and the Iberian peninsula (Hymenoptera: Apoidea). Systematic Entomology 8(1): 29–62. https://doi.org/10.1111/j.1365-3113.1983.tb00466.x

Engelstädter J (2008) Constraints on the evolution of asexual reproduction. BioEssays 30(11–12): 1138–1150. https://doi.org/10.1002/bies.20833

Engelstädter J (2017) Asexual but not clonal: evolutionary processes in automictic populations. Genetics 206(2): 993–1009. https://doi.org/10.1534/genetics.116.196873

Fougeyrollas R, Dolejšová K, Sillam-Dussès D, Roy V, Poteaux C, Hanus R, Roisin Y (2015) Asexual queen succession in the higher termite *Embiratermes neotenicus*. Proceedings of the Royal Society B: Biological Sciences 282(1809): e20150260. https://doi.org/10.1098/rspb.2015.0260

Fujita MK, Singhal S, Brunes TO, Maldonado JA (2020) Evolutionary dynamics and consequences of parthenogenesis in vertebrates. Annual Review of Ecology, Evolution, and Systematics 51(1): 191–214. https://doi.org/10.1146/annurev-ecolsys-011720-114900

Gerber HS, Klostermeyer EC (1970) Sex control by bees: A voluntary act of egg fertilization during oviposition. Science 167(3914): 82–84. https://doi.org/10.1126/science.167.3914.82

Gokhman VE, Kuznetsova VG (2018) Parthenogenesis in Hexapoda: Holometabolous insects. Journal of Zoological Systematics and Evolutionary Research 56(1): 23–34. https://doi.org/10.1111/jzs.12183

Goudie F, Oldroyd BP (2014) Thelytoky in the honey bee. Apidologie 45(3): 306–326. https://doi.org/10.1007/s13592-013-0261-2

Goudie F, Oldroyd BP (2018) The distribution of thelytoky, arrhenotoky and androgenesis among castes in the eusocial Hymenoptera. Insectes Sociaux 65(1): 5–16. https://doi.org/10.1007/s00040-017-0597-0

Groom SVC, Rehan SM (2018) Climate-mediated behavioural variability in facultatively social bees. Biological Journal of the Linnean Society 125(1) 165–170. https://doi.org/10.1093/biolinnean/bly101

Hamilton WD (1964) The genetical evolution of social behaviour. I. Journal of Theoretical Biology 7(1): 1–16. https://doi.org/10.1016/0022-5193(64)90038-4

Heinze J (2008) The demise of the standard ant (Hymenoptera: Formicidae). Myrmecological News 11: 9–20.

Hörandl E, Bast J, Brandt A, Scheu S, Bleidorn C, Cordellier M, Nowrousian M, Begerow D, Sturm A, Verhoeven K (2020) Genome evolution of asexual organisms and the paradox of sex in eukaryotes. Evolutionary Biology–A Transdisciplinary Approach: 133–167. https://doi.org/10.1007/978-3-030-57246-4_7

Kooi CJ van der, Matthey-Doret C, Schwander T (2017) Evolution and comparative ecology of parthenogenesis in haplodiploid arthropods. Evolution Letters 1(6): 304–316. https://doi.org/10.1002/evl3.30

Liegeois M, Sartori M, Schwander T (2021) Extremely widespread parthenogenesis and a trade-off between alternative forms of reproduction in mayflies (Ephemeroptera). Journal of Heredity 112(1): 45–57. https://doi.org/10.1093/jhered/esaa027

Lorenzo-Carballa MO, Cordero-Rivera A (2009) Thelytokous parthenogenesis in the damselfly *Ischnura hastata* (Odonata, Coenagrionidae): Genetic mechanisms and lack of bacterial infection. Heredity 103(5): [Article] 5. https://doi.org/10.1038/hdy.2009.65

Mikát M, Benda D, Korittová C, Mrozková J, Reiterová D, Waldhauserová J, Brož V, Straka J (2020) Natural history and maternal investment of *Ceratina cucurbitina*, the most common European small carpenter bee, in different European regions. Journal of Apicultural Research: 1–12. https://doi.org/10.1080/00218839.2020.1828235

Mikát M, Fraňková T, Benda D, Straka J (2022) Evidence of sociality in European small carpenter bees (*Ceratina*). Apidologie 53(2): 1–18. https://doi.org/10.1007/s13592-022-00931-8

Mikát M, Janošík L, Černá K, Matoušková E, Hadrava J, Bureš V, Straka J (2019) Polyandrous bee provides extended offspring care biparentally as an alternative to monandry based eusociality. Proceedings of the National Academy of Sciences 116(13): 6238–6243. https://doi.org/10.1073/pnas.1810092116

Mikát M, Matoušková E, Straka J (2021) Nesting of *Ceratina nigrolabiata*, a biparental bee. Scientific Reports 11(1): [Article] 1. https://doi.org/10.1038/s41598-021-83940-4

Mueller UG (1991) Haplodiploidy and the evolution of facultative sex ratios in a primitively eusocial bee. Science 254(5030): 442–444. https://doi.org/10.1126/science.254.5030.442

Neiman M, Meirmans S, Meirmans P (2009) What can asexual lineage age tell us about the maintenance of sex? Annals of the New York Academy of Sciences 1168(1): 185–200. https://doi.org/10.1111/j.1749-6632.2009.04572.x

Neiman M, Schwander T (2011) Using parthenogenetic lineages to identify advantages of sex. Evolutionary Biology *38*(2): 115–123. https://doi.org/10.1007/s11692-011-9113-z

Normark BB (2003) The evolution of alternative genetic systems in insects. Annual Review of Entomology 48(1): 397–423. https://doi.org/10.1146/annurev.ento.48.091801.112703

Pearcy M, Hardy O, Aron S (2006) Thelytokous parthenogenesis and its consequences on inbreeding in an ant. Heredity 96(5): [Article] 5. https://doi.org/10.1038/sj.hdy.6800813

Rabeling C, Kronauer DJ (2013) Thelytokous parthenogenesis in eusocial Hymenoptera. Annual Review of Entomology 58: 273–292. https://doi.org/10.1146/annurev-ento-120811-153710

Rehan SM (2020) Small carpenter bees (*Ceratina*). In: Starr CK (Ed.) Encyclopedia of Social Insects. Springer International Publishing, 4 pp. https://doi.org/10.1007/978-3-319-90306-4_106-1

Rehan SM, Richards MH (2010) Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). The Canadian Entomologist 142(1): 65–74. https://doi.org/10.4039/n09-056

Rehan SM, Richards MH, Schwarz MP (2009) Evidence of social nesting in the *Ceratina* of Borneo (Hymenoptera: Apidae). Journal of the Kansas Entomological Society 82(2): 194–209. https://doi.org/10.2317/JKES809.22.1

Rehan S, Schwarz M (2015) A few steps forward and no steps back: Long-distance dispersal patterns in small carpenter bees suggest major barriers to back-dispersal. Journal of Biogeography 42(3): 485–494. https://doi.org/10.1111/jbi.12439

Rey O, Loiseau A, Facon B, Foucaud J, Orivel J, Cornuet J-M, Robert S, Dobigny G, Delabie JHC, Mariano CDSF, Estoup A (2011) Meiotic recombination dramatically decreased in thelytokous queens of the little fire ant and their sexually produced workers. Molecular Biology and Evolution 28(9): 2591–2601. https://doi.org/10.1093/molbev/msr082

Rousset F (2020) Genepop Version 4.7. 0. Institut des Sciences de l'Evolution de Montpellier, Université de Montpellier.

Ryskov AP, Osipov FA, Omelchenko AV, Semyenova SK, Girnyk AE, Korchagin VI, Vergun AA, Murphy RW (2017) The origin of multiple clones in the parthenogenetic lizard species *Darevskia rostombekowi*. PLoS ONE 12(9): e0185161. https://doi.org/10.1371/journal.pone.0185161

Sakagami SF, Maeta Y (1977) Some presumably presocial habits of Japanese *Ceratina* bees, with notes on various social types in Hymenoptera. Insectes Sociaux 24(4): 319–343. https://doi.org/10.1007/BF02223784

Schwander T, Crespi BJ (2009) Multiple direct transitions from sexual reproduction to apomictic parthenogenesis in *Timema* stick insects. Evolution 63(1): 84–103. https://doi.org/10.1111/j.1558-5646.2008.00524.x

Shell WA, Rehan SM (2019) Invasive range expansion of the small carpenter bee, *Ceratina dentipes* (Hymenoptera: Apidae) into Hawaii with implications for native endangered species displacement. Biological Invasions 21(4): 1155–1166. https://doi.org/10.1007/s10530-018-1892-z

Shukla S, Shilpa MC, Gadagkar R (2013) Virgin wasps develop ovaries on par with mated females, but lay fewer eggs. Insectes Sociaux 60(3): 345–350. https://doi.org/10.1007/s00040-013-0299-1

Simon J-C, Delmotte F, Rispe C, Crease T (2003) Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. Biological Journal of the Linnean Society 79(1): 151–163. https://doi.org/10.1046/j.1095-8312.2003.00175.x

Slobodchikoff CN, Daly HV (1971) Systematic and evolutionary implications of parthenogenesis in the Hymenoptera. American Zoologist 11(2): 273–282. https://doi.org/10.1093/icb/11.2.273

Snelling RR (2003) Bees of the Hawaiian Islands, exclusive of *Hylaeus* (*Nesoprosopis*)(Hymenoptera: Apoidea). Journal of the Kansas Entomological Society: 342–356.

Stenberg P, Saura A (2009) Cytology of Asexual Animals. In: Schön I, Martens K, Dijk P (Eds) Lost Sex. Springer, Dordrecht, 63–74. https://doi.org/10.1007/978-90-481-2770-2_4

Stubblefield JW, Seger J (1994) Sexual Dimorphism in the Hymenoptera. The Differences Between the Sexes. Cambridge University Press, Cambridge, 71–103.

Terzo M (1998) Annotated list of the species of the genus *Ceratina* (Latreille) occuring in the Near East, with descriptions of new species (Hymenoptera: Apoidea: Xylocopinae). Linzer Biologische Beiträge 30(2): 719–743.

Terzo M, Iserbyt S, Rasmont P (2007) Révision des Xylocopinae (Hymenoptera: Apidae) de France et de Belgique. Annales de La Société Entomologique de France 43: 445–491. https://doi.org/10.1080/00379271.2007.10697537

Terzo M, Rasmont P (2004) Biogéographie et systématique des abeilles rubicoles du genre *Ceratina* Latreille au Turkestan (Hymenoptera, Apoidea, Xylocopinae). Annales de La Société Entomologique de France (N.S.) 40(2): 109–130. https://doi.org/10.1080/00379271.2004.10697410

Terzo M, Rasmont P (2011) Atlas of the European Bees: Genus *Ceratina*. Atlas Hymenoptera – Atlas of the European Bees – STEP Project. http://www.atlashymenoptera.net/page.asp?id=192

Thierry L (2013) Adaptive significance and long-term survival of asexual lineages 2. Evolutionary Biology 40(3): 450–460. https://doi.org/10.1007/s11692-012-9219-y

Tsutsui Y, Maeto K, Hamaguchi K, Isaki Y, Takami Y, Naito T, Miura K (2014) Apomictic parthenogenesis in a parasitoid wasp *Meteorus pulchricornis*, uncommon in the haplodiploid order Hymenoptera. Bulletin of Entomological Research 104(3): e307. https://doi.org/10.1017/S0007485314000017

Tvedte ES, Logsdon JM, Forbes AA (2019) Sex loss in insects: Causes of asexuality and consequences for genomes. Current Opinion in Insect Science 31: 77–83. https://doi.org/10.1016/j.cois.2018.11.007

Vorburger C (2014) Thelytoky and sex determination in the Hymenoptera: Mutual constraints. Sexual Development 8(1–3): 50–58. https://doi.org/10.1159/000356508

Wenseleers T, Van Oystaeyen A (2011) Unusual modes of reproduction in social insects: Shedding light on the evolutionary paradox of sex. BioEssays 33(12): 927–937. https://doi.org/10.1002/bies.201100096

## Supplementary material 1

**Dataset**

Authors: Michael Mikát, Jakub Straka

Data type: occurences, genetic

Explanation note: Primary data used for paper Genetic evidence for parthenogenesis in small carpenter bee, *Ceratina dallatoreana* in its native distribution area. Dataset contains information about lotaions and dates of collected samples, lenght of microsatellite loci and infromation to which analyses was sample included.

Link: https://doi.org/10.3897/jhr.95.87165.suppl1

## Supplementary material 2

**Faunistic notes and microsatellite primers**
Authors: Michael Mikát, Jakub Straka
Data type: genetic, faunistic
Explanation note: 1) notes about distribution of species *C. dallatoreana* 2) Features of microsatellite loci of *C. dallatoreana* 3) Features of successfully amplified microsatellites for *C. dallatoreana* 4) frequencies of alleles of microsatellite loci of *C. dallatoreana* 5) Comparison between expected and observed proportion of heterozygotes on reduced dataset from Lefkara (Cyprus) and Georgiopoli (Crete).
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/jhr.95.87165.suppl2