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Androgenesis is a maternal trait in the invasive ant *Wasmannia auropunctata*

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Androgenesis is the production of an offspring containing exclusively the nuclear genome of the fathering male via the maternal eggs. This unusual mating system is generally considered a male trait, giving to androgenetic males a substantial fitness advantage over their sexually reproducing relatives. We here provide the first empirical study of the evolutionary outcomes of androgenesis in a haplo-diploid organism: the invasive ant *Wasmannia auropunctata*. Some of the populations of this species have a classical haplo-diploid sexual mating system. In other populations, females and males are produced through parthenogenesis and androgenesis, respectively, whereas workers are produced sexually. We conducted laboratory reciprocal-cross experiments with reproductive individuals from both types of populations and analysed their progenies with genetic markers, to determine the respective contribution of males and females to the production of androgenetic males. We found that androgenesis was a parthenogenetic female trait. A population genetic study conducted *in natura* confirmed the parthenogenetic female origin of androgenesis, with the identification of introgression events of sexual male genotypes into androgenetic/parthenogenetic lineages. We argue that by producing males via androgenesis, parthenogenetic queen lineages may increase and/or maintain their adaptive potential, while maintaining the integrity of their own genome, by occasionally acquiring new male genetic material and avoiding inbreeding depression within the sexually produced worker cast.

1. Introduction

Most living organisms reproduce through sexual reproduction [1], which can be seen as a shared enterprise into which two unrelated individuals invest resources. This situation favours individuals that manipulate their mates to maximize their own fitness, even if this has negative consequences for their partners, creating so-called 'sexual conflict' [2]. All aspects of investment in offspring are likely to be subject to conflict, from gamete size (i.e. anisogamy) to all subsequent investment in the zygote, gestation or parental care [3]. This has led to the emergence of various mating systems in which each of the partners tries to maximize its own advantage [4].

Among the variety of mating systems, androgenesis is probably the least studied and the most puzzling. Androgenesis is the production of an offspring containing exclusively the nuclear genome of the fathering male via the maternal eggs. Androgenesis has traditionally been regarded as a male trait, giving to androgenetic males a substantial fitness advantage over their sexually reproducing relatives [5]. Androgenesis has, to date, been identified as the main form of reproduction in a very small number of species from four distinct taxa: the hermaphrodite Saharan cypress tree *Cupressus dupreziana* [6], four hermaphrodite species of freshwater clams from the genus *Corbicula* [7], a hybrid complex of *Bacillus* stick insects [8,9], and three haplo-diploid ant species: *Wasmannia auropunctata* [10,11], *Vollenhovia emeryi* [12] and *Paratrechina longicornis* [13].

Theoretical models predict that androgenesis mutations in sexually reproducing diploid organisms will rapidly spread and become fixed, leading the populations concerned to extinction [5]. Extinction may be avoided in

populations that retain female reproductive capacity [5]; for instance, when androgenesis results from hybridization between sexually reproducing related species, as in the *Bacillus* stick insect hybrid complex [9,14], or through hermaphroditism, as for the cypress tree *C. dupreziana* and the clams of the genus *Corbicula*. Recent empirical studies on *Corbicula* have suggested that androgenesis, associated with hermaphroditism, may have played an important role in the invasion of some androgenetic lineages, particularly as androgenetic sperm can parasitize the maternal gametes of other lineages [7]. However, we are aware of no theoretical or empirical studies investigating the evolutionary outcomes of androgenesis in haplo-diploid organisms, such as in ants.

In the previously mentioned ant species, males are produced by androgenesis in certain populations in which female reproductive individuals (i.e. queens) reproduce via parthenogenesis and workers (i.e. sterile individuals) are produced by sexual reproduction [10,12,13]. In *W. auropunctata*, this unusual mating system is not induced by any of the most common endosymbiotic manipulators [15] and appears instead to be genetically determined [16]. Foucaud *et al.* [10] demonstrated that *W. auropunctata* populations displaying a parthenogenesis–androgenesis system (hereafter referred to as ‘clonal’ populations, for simplicity) have recurrently emerged from ancestral classical haplo-diploid sexual populations (hereafter referred to as ‘sexual’ populations), in which females (i.e. queens and sterile workers) develop from diploid fertilized eggs and males develop from haploid unfertilized eggs (arrhenotoky). Type of mating system is strongly associated with ecological features, including invasive status, in *W. auropunctata* populations [17]. Sexual populations, with low nest and worker densities, generally occur in natural habitats (i.e. primary tropical forests), whereas clonal populations are mostly found in disturbed habitats and display invasive characteristics (i.e. high worker and nest densities [17,18]). This suggests that clonal and sexual populations occupy different ecological niches, and that human disturbance commonly drives habitats towards ecological conditions favouring the establishment of invasive clonal populations.

Two main hypotheses may be given for explaining the mechanisms of androgenesis in some populations of *W. auropunctata*. First, androgenesis may result from the expected struggle between females and males for access to the egg [2], in which case androgenesis would be a male response to female parthenogenesis and would therefore be a male trait. This hypothesis, first proposed by Fournier *et al.* [11], has, however, been called into doubt by Foucaud *et al.* [10]. The latter authors suggested that androgenesis may instead be a parthenogenetic female trait to increase their own fitness by (i) allowing the reproduction of females and males from the same cohort without inbreeding depression effects in their sexually produced workers, and (ii) fixing an adapted male genome in the parthenogenetic–androgenetic lineage for the production of workers via sexual reproduction. The two above hypotheses remained to be rigorously tested using appropriate laboratory-controlled experimental designs.

We here investigated the respective roles of males and females in androgenesis, by conducting a laboratory-controlled reciprocal-cross experiment with reproductive individuals (i.e. females and males) from sexual and clonal populations, using genetic markers to determine how their progenies were produced, focusing particularly on male offspring. Under the hypothesis that androgenesis is a male trait [11], androgenetic

males from clonal populations are expected to produce androgenetic sons regardless of whether their female mates originate from clonal or sexual populations. On the contrary, if androgenesis is a parthenogenetic female trait, as hypothesized in earlier studies [10,17,19], then parthenogenetic females are expected to produce androgenetic sons regardless of whether they were fertilized by males from sexual populations or from other unrelated clonal populations. Finally, we also conducted a population genetic study *in natura* in a localized geographical zone where clonal and sexual populations live in close proximity, to detect possible natural crosses between reproductive individuals from sexual and clonal populations, and to determine the directionality of androgenesis in wild populations.

2. Material and methods

(a) Sampling and cross-breeding experiments

We sampled nine populations (eight in French Guiana and one in Israel), consisting of approximately 20–30 fertilized queens and 5000–10 000 workers (see the electronic supplementary material, table S1). Based on previous genetic studies of populations collected in the field at the same locations [17,20,21], we predicted that four of the collected populations would be sexual and the other five clonal. The mode of reproduction of each population sampled was confirmed *a posteriori*, by genetic analyses on a subset of reproductive individuals.

Ants were maintained at constant temperature and humidity (25°C; 70% RH; 12 L : 12 D cycle) and fed *ad libitum* with *Ephestia* eggs and a honey–yeast–water solution. These populations were bred as source populations for the production of males and virgin queens, which were used for the reciprocal cross-breeding experiments. The production of reproductive individuals in laboratory source populations of *W. auropunctata* is laborious and stochastic. Crosses were thus strongly constrained by the synchronous production of males and females in these source populations with different mating systems. We crossed queens from sexual populations with males from clonal populations ($F_S \times M_C$ crosses), and females from clonal populations with males from either sexual populations ($F_C \times M_S$ crosses) or from unrelated clonal populations ($F_C \times M_C$ crosses). We set up 90 crosses between reproductive individuals from the various clonal and sexual populations, as follows. One to six alate (i.e. virgin) queens from a given population were placed in an artificial nest, isolated in sealed and meshed boxes with one to 10 males, and without workers, until at least some of the queens lost their wings (queens lose their wings only after mating or with age). All the potential fathering males involved in the crosses were collected and stored in ethanol for subsequent genetic analyses.

Each freshly fertilized (i.e. wingless) queen was then placed in a separate box, with 50 workers from her source population, and food was supplied *ad libitum*. The resulting monogynous lineages were then reared under the same laboratory conditions as the source populations and checked twice weekly. The reproductive progeny (i.e. daughters and sons) produced by each monogynous lineage was collected and stored in ethanol until the queen died or for a maximum of 621 days. At the end of the experiments, all mothering queens and their workers were collected and stored in ethanol for subsequent genetic analyses.

(b) Genetic analyses

(i) Cross effectiveness and identification of the fathering males

From the 90 initial crosses, we obtained 34 monogynous (wingless) lineages, 27 of which produced female and male

reproductive progeny, and in some cases gynandromorphs (i.e. reproductive individuals with both female and male phenotypic characters). We checked the effectiveness of the 27 successful crosses and identified by genotyping, at 12 microsatellite markers, the fathering males, all males potentially involved in the cross, the queen and eight of the adult workers collected at the end of the experiment. DNA was extracted from each individual and microsatellite genotypes were obtained as described by Fournier *et al.* [11,22]. For a given monogynous cross, the multilocus genotype of each worker was compared with the genotypes of the queen and of all males potentially involved in the cross. A male was identified as the effective fathering male if its allele at each locus was compatible with the paternal allele identified from the genotypes of the eight workers.

(ii) Determination of the mode of offspring production

In total, 398 reproductive offspring (178 males and 220 females) from 22 of the 27 successful crosses underwent a genetic analysis of the same 12 microsatellite markers. The progenies of the other five crosses were not included in the analyses, because the queens involved in these crosses produced too few reproductive individuals. The numbers of male and female offspring genotyped for each cross are given in the electronic supplementary material, table S2. Males were considered to have been produced by androgenesis if their haploid multilocus genotype was identical to that of the fathering male, and by arrhenotoky if their haploid multilocus genotype contained only maternal alleles. Males were considered mosaic polyploids when they displayed different levels of ploidy (i.e. haploidy, diploidy and/or triploidy) at the 12 analysed microsatellite loci. Females were considered to have been produced by parthenogenesis if their diploid multilocus genotype was identical to that of the mothering queen, and by sexual reproduction if their diploid genotype included an allele inherited from each parent, at each of the 12 loci considered. Females were considered mosaic polyploids when they displayed different levels of ploidy at the 12 analysed microsatellite loci.

(iii) Determination of the mode of transmission of the mitochondrial genome as a marker of cytoplasmic material

We identified seven monogynous lineages being produced by the three different types of cross and for which the queen and the fathering male were known to display different haplotypes for a fragment of the mtDNA gene, cytochrome oxidase I (*COI*). We then characterized the mtDNA haplotypes transmitted to 37 male and female reproductive offspring in the next generation of these seven lineages (see the electronic supplementary material, table S3 for details). The 520 bp fragment of the mitochondrial *COI* gene was amplified by PCR for each individual, as described by Foucaud *et al.* [10]. Individual electropherograms were checked for potential errors with SEQSCAPE software (Applied Biosystems). Aligned sequences were analysed visually by comparing the sequence of each reproductive offspring with those of its parents.

(iv) Genetic analyses of natural populations

A previous population genetics study [10] demonstrated that *W. auropunctata* clonal and sexual populations were not separate evolutionary units and provided evidence that parthenogenetic lineages of *W. auropunctata* arise recurrently from sexual populations. It did not evaluate, however, the possibility that (androgenetic) males of parthenogenetic lineages could originate through introgression from neighbouring sexual populations. Such events, if identified, would represent *in natura* evidence that androgenesis is a maternal trait. To tackle this question, we conducted a population genetics study in French Guiana, along

a forest track (i.e. in a human-disturbed habitat), where *W. auropunctata* populations were expected to be mostly clonal, and for surrounding populations established in the tropical primary forest (i.e. in a natural habitat), where populations were expected to be mostly sexual [17,18] (electronic supplementary material, figure S1). We were particularly interested in assessing whether parthenogenetic queens of natural clonal populations along the forest track could produce androgenetic sons from fathering males originating from surrounding sexual populations. In total, 73 queens and 50 males (five males and the spermathecal contents of 45 queens) were collected from 19 populations established along the forest track, and 20 queens and 25 males (13 males and the spermathecal contents of 12 queens) were collected from four populations established in the tropical forest (see the electronic supplementary material, figure S1). DNA was extracted from each individual and genotyped at the same 12 microsatellite loci as above. Population delineation was inferred through behavioural interaction tests between 261 pairs of nests, as described by Foucaud *et al.* [17]. The clonal or sexual mode of reproduction of each queen and male was inferred by visually inspecting individual genotypes and with an in-house program identifying identical multilocus genotypes. A custom-made program was used to reconstruct the genetic relationship between parental genotypes through a dendrogram representation, with individual genotypes as tips, based on the neighbour-joining (NJ) algorithm [23]. The genetic distance used to construct the dendrograms was a variant of Chakraborty & Jin's [24] allele-shared distance, as defined by Fournier *et al.* [11].

3. Results and discussion

Genotypic data from the laboratory reciprocal-cross experiments show that all male offspring ($n = 107$) from the crosses involving queens from sexual populations and males from clonal populations ($F_S \times M_C$ crosses) developed from unfertilized eggs through arrhenotoky, as in standard sexual populations (figure 1a). Androgenetic males from clonal populations are thus incapable of producing a male offspring via androgenesis when fertilizing females from sexual populations. Furthermore, the queens resulting from these crosses ($n = 60$) were produced exclusively by sexual reproduction (figure 1b). Thus, males from clonal populations clearly have no influence on the way in which queens from sexual populations produce their reproductive offspring (i.e. sexual reproduction and arrhenotoky to produce queens and males, respectively).

We found that queens from clonal populations produced haploid male offspring almost exclusively by androgenesis (i.e. all but one case; figure 1a), regardless of whether they were fertilized by males from sexual populations ($F_C \times M_S$ crosses) or from other unrelated clonal populations ($F_C \times M_C$ crosses). This result echoes those of previous studies indicating that queens from clonal populations seldom produce arrhenotokous male progeny either *in natura* [19] or in laboratory conditions [16]. The single arrhenotokous male produced by a parthenogenetic queen in this study therefore probably did not result from mating with a male from a sexual population. Consistent with this hypothesis, the parthenogenetic queen that produced the arrhenotokous male also produced eight androgenetic males ($F_C \times M_S$ cross $C_2 \times S_1$ -IV in the electronic supplementary material, table S4).

Overall, our laboratory results indicate that androgenesis in *W. auropunctata* is not a male trait, but instead is a parthenogenetic female trait. In agreement with this, we identified at least two introgression events of a sexual male genome

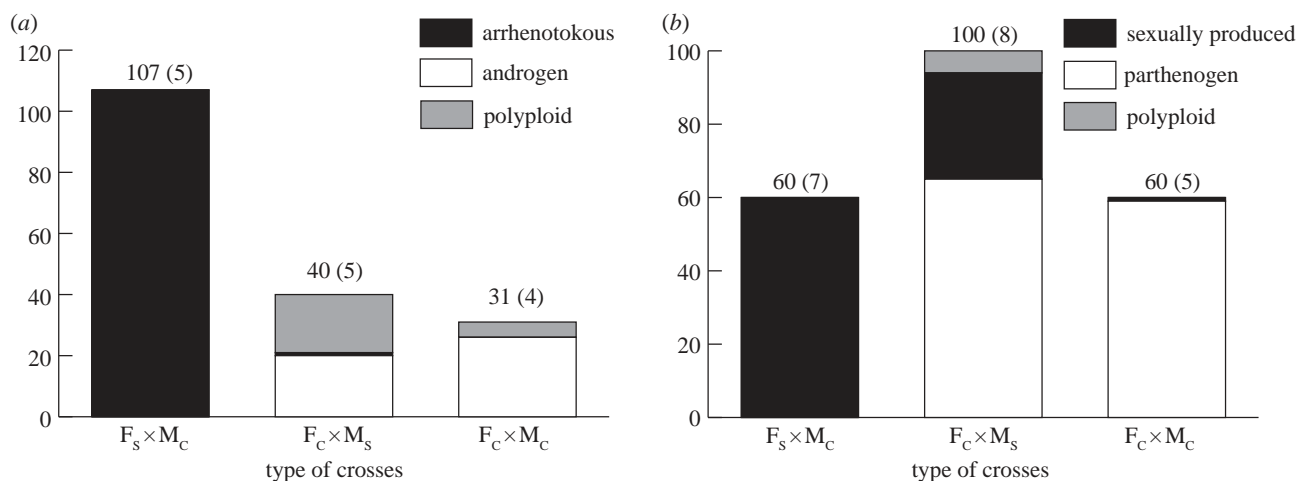


Figure 1. Number and type of (a) male and (b) queen progeny produced by the 22 laboratory crosses analysed genetically. The number of each genotyped progeny is indicated above the vertical bars and the numbers of crosses involved are indicated in brackets. $F_s \times M_c$: sexual queen mated with clonal male. $F_c \times M_s$: clonal queen mated with sexual male. $F_c \times M_c$: clonal queen mated with clonal male. In all the crosses studied, the genotyped workers were all produced through sexual reproduction involving a single fathering male. See the electronic supplementary material, table S4 for a detailed description of results for each cross.

within parthenogenetic lineages *in natura*, in a geographical zone where sexual and clonal populations live in close proximity (see the electronic supplementary material, figure S1). The genotypes of males fathering two clonal populations each characterized by distinct female parthenogenetic lineages were very similar to the male genotypes typical of the neighbouring sexual populations (figure 2). These males therefore probably originated from such sexual populations (see Tk03_M and Tk04_S male genotypes in figure 2). As in our $F_c \times M_s$ laboratory-controlled crosses, these males, probably produced by arrhenotoky, fathered clonal males when mated with clonal females. The *in natura* finding that parthenogenetic females may produce androgenetic sons from fathering males originating from sexual populations extends beyond laboratory conditions the conclusion that androgenesis is a female trait in *W. auropunctata*.

The mutational origin of androgenesis remains largely unknown. The hypothesis of a mutation inducing androgenesis appearing in the queen lineage independently of the parthenogenesis mutation seems unlikely, for at least three reasons: (i) a parthenogenetic queen producing only arrhenotokous males has never been identified; (ii) androgenesis has never been observed in sexual populations; and (iii) this scenario is not parsimonious, as it requires two independent evolutionary changes in the same parthenogenetic queen lineage. The hypothesis of androgenesis emerging simultaneously in queens as a by-product of parthenogenesis is more parsimonious. It requires a single evolutionary change for the emergence of both parthenogenesis and androgenesis, and better explains the tight association between the two genetic systems observed in natural populations [10]. Furthermore, Rey *et al.* [20] recently demonstrated that queen parthenogenesis involves meiosis (automictic parthenogenesis) with the central fusion of oocytes with a substantial decrease in genetic recombination during meiosis. Mechanistically, recombination is of prime importance for correct chromosome segregation during meiosis [25], and major decreases in recombination rates may lead to the missegregation of chromosomes and, consequently, to the production of aneuploid eggs [25,26]. This would render a mechanism of maternal genome exclusion easier and might even lead directly to the production of empty eggs, which could be fertilized by males to produce androgenetic sons. It is worth

noting that we found a traditional female transmission of the mitochondrial genome whatever the nature of the offspring (i.e. arrhenotokous or androgenetic male and sexually produced or parthenogenetic female) and the type of cross (see the electronic supplementary material, table S3). As a result, although emptied of their maternal nuclear genomic contents, eggs prone to develop into androgenetic males still contain the maternal mitochondrial genome.

Irregularities in the meiotic machinery of parthenogenetic queens may also lead to the occasional production of degenerate progeny, such as mosaic polyploids (i.e. individuals containing populations of cells characterized by different levels of ploidy) and/or gynandromorphs (i.e. individuals displaying both female and male phenotypic characters [25]). Consistent with this hypothesis, both mosaic polyploids and gynandromorphs were detected in the progenies of *W. auropunctata* parthenogenetic queens, whether fertilized by males from clonal ($F_c \times M_c$) or sexual ($F_c \times M_s$) populations (figures 1 and 3; see also the electronic supplementary material, figure S2 for an illustration of gynandromorphy). The presence of mosaic polyploid males probably stemmed from the development of aneuploid fertilized eggs in which the exclusion of the maternal genome was only partial, although this remains to be thoroughly demonstrated. By contrast, no irregular progeny were observed in crosses involving queens from sexual populations. Gynandromorphs have been reported *in natura* in invasive (clonal) populations of *W. auropunctata* from Brazil and Israel (J. H. D. Delabie 2009, personal communication; Y. B. Mordehai 2009, personal communication).

The production of males through androgenesis by parthenogenetic females has far-reaching evolutionary implications. First, androgenesis allows reproduction to occur between females and males from the same cohort without inbreeding depression effects in their sexually produced workers, as previously suggested by Foucaud *et al.* [10] and by Pearcy *et al.* [13] for the ant *P. longicornis*. This outcome may be crucial in the particular context of invasions [10,17]. Second, this mechanism maintains high levels of heterozygosity and/or beneficial genetic combinations over time in the sexually produced worker offspring. In agreement with this, Foucaud *et al.* [16,27] found that heterozygosity, in workers from clonal populations, is on average higher than that in sexual

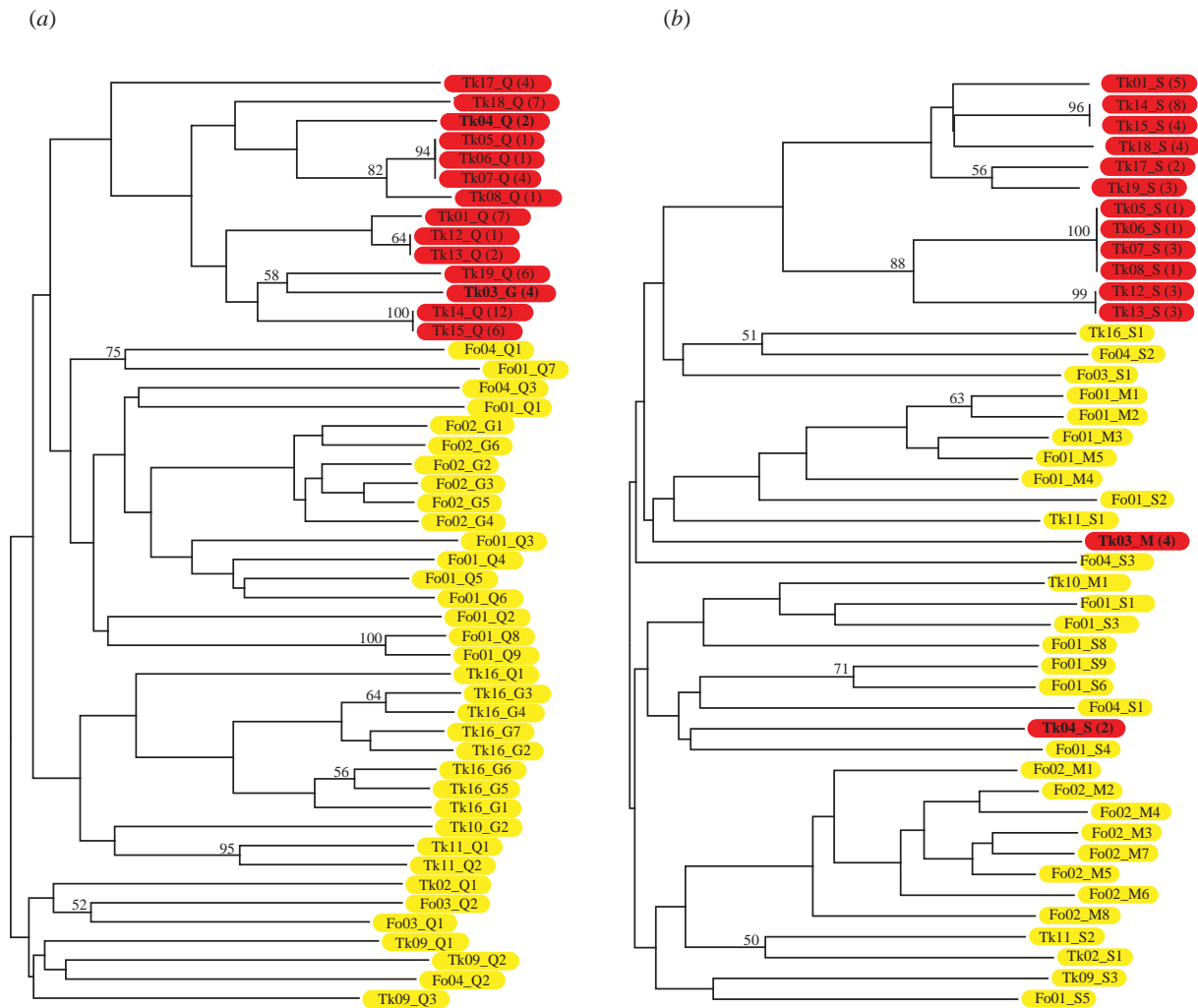


Figure 2. NJ dendrograms of the genetic distances at microsatellite markers between (a) queens and (b) males from sexual (yellow) and clonal (red) populations studied *in natura*. The sampled natural area is described in the electronic supplementary material, figure S1. Individuals are labelled as follows: habitat of origin (Tk: forest track; Fo: primary tropical forest in the vicinity of the forest track), population number, gender (Q: queen, G: gyne, M: male, S: spermatocae) and individual number. The number of genetically identical individuals genotyped in clonal populations is indicated in parentheses. Bootstrap values for nodes (computed from 1000 replicates; bootstrapping over loci) are shown only when greater than 50%. Note that low bootstrap values are usually obtained for trees with individuals as tips.

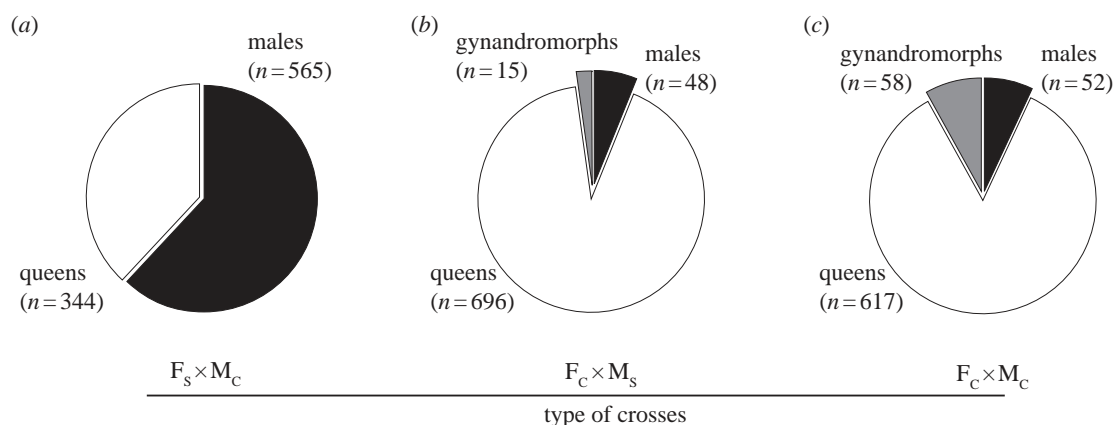


Figure 3. Number of males, queens and gynandromorphs produced by the 27 laboratory crosses studied. (a) $F_S \times M_C$: sexual queen mated with clonal male. (b) $F_C \times M_S$: clonal queen mated with sexual male. (c) $F_C \times M_C$: clonal queen mated with clonal male.

populations. Finally, the theory predicts that diploid asexual lineages are destined to die out, owing to their limited ability to generate genomic innovation through the syngamy of two meiotically recombined gametes [28,29] and because of the reproductive isolation from males provided by parthenogenesis [30]. This evolutionary cost of asexuality may

be reduced in *W. auropunctata* parthenogenetic female lineages given their ability to occasionally reproduce with males originating from distinct evolutionary lineages. The patency of parthenogenetic lineages to the introgression of new male genomes allows the creation of genetic diversity among parthenogenetic–androgenetic lineages at the level of the sexually

produced worker offspring. In a changing environment, the production of androgenetic males from a new male genome by a parthenogenetic queen may allow selection to fix a better-adapted genomic combination, including the new male genome and the same conserved parthenogenetic genome for the production of workers. In eusocial species, queens are generally confined to favourable conditions within the nest, and selection is more likely to act on workers, which have to face various biotic and abiotic environmental factors during foraging [31]. We therefore argue that renewing the male genomic material used for the production of sexually produced workers within the lineage might be an unprecedented manner for parthenogenetic queens to preserve (if not increase) their adaptive potential and, hence, their lifetime, while maintaining the integrity of their own genome.

The benefit of androgenesis for parthenogenetic queens would be increased by these queens preferentially allocating their resources to the production of female, rather than male, reproductive individuals [32]. From an inclusive fitness perspective, queens reproducing by thelytokous parthenogenesis are expected to produce a more female-biased sex ratio than sexually reproducing queens. This is because clonal queens are related by 1 to their own daughters and by 0 to the androgenetic males, whereas sexually reproducing queens are related by 0.5 to both offspring sexes. Consistent with this, we found that parthenogenetic queens produced 93.6% and 92.2% reproductive females in the $F_C \times M_S$ and the $F_C \times M_C$ crosses, respectively, whereas sexually reproducing queens produced 37.8% reproductive females when fertilized by males from clonal populations ($p < 0.01$; figure 3; electronic supplementary material, table S2). It is worth noting that the more female-biased sex ratio observed in the offspring of parthenogenetic compared with sexually reproducing queens may also depend on proximal factors such as the number of enucleated eggs, and hence of potentially (androgenetic) males produced by parthenogenetic queens, which is likely to be relatively low, although this remains an open question.

Finally, we found that about one-third (i.e. 29%) of the queen daughters were produced sexually in the $F_C \times M_S$ crosses. This proportion is substantially higher than that observed in crosses involving reproductive individuals from unrelated clonal populations (i.e. 1.67% in this case; $\chi^2 = 13.53$; p -value = 2.4×10^{-4} ; figure 1; electronic supplementary material, table S4) or reproductive individuals from the same clonal population [16]. The production of female reproductive individuals through sexual reproduction

by parthenogenetic queens therefore appears to be, at least partly, a consequence of their fertilization by males from sexual populations. The exact causes of the production of queens via sexual reproduction by parthenogenetic queens when fertilized by males originating from sexual populations remain unexplained, however.

In conclusion, this study demonstrates that in the haplo-diploid species *W. auropunctata*, androgenesis is a parthenogenetic female trait. Thus, in this species, androgenesis does not conform to the general view of a male trait, sometimes considered as an egg parasitism strategy by males [7]. The mating system uncovered in *W. auropunctata*, although probably resulting from meiotic irregularities, allows parthenogenetic queens to preserve, if not increase, their own fitness through their sexually produced workers, while keeping their own genomes intact in the offspring. Interestingly, this androgenetic—parthenogenetic system has proved particularly successful in the context of invasions of human-disturbed habitats in which environmental conditions may change abruptly, such as the human-disturbed habitats invaded by *W. auropunctata*, which are generally hotter and drier than those in the original range of this species [18]. Consistent with this, all three ant species in which androgenesis occurs are recognized as invasive species [13,17,33]. However, the androgenetic—parthenogenetic system seems to be largely overwhelmed by the classical sexual reproduction system in natural habitats (i.e. primary tropical forest) of *W. auropunctata*, in which more complex biotic interactions occur [17,18] and more rapid evolutionary changes of various types, mediated by recombination and syngamy at each generation, are probably required [34].

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Data accessibility. Microsatellite datasets for analysing the offspring production of laboratory reciprocal-crosses are available at Dryad Digital repository (doi:10.5061/dryad.fg32t). Mitochondrial *COI* sequences for the determination of the mode of transmission of the mitochondrial genome are available at GenBank under accession nos. KF018288–KF018338.

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