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Primary sex ratio regulation by queens in ants (Formicidae) and other social Hymenoptera

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Abstract

Sex allocation theory describes how parents should bias investment in either sons or daughters when each gives a different fitness return. Over the past decades, social Hymenoptera have increasingly been used as models for testing the predictions of sex ratio theory. Their haplodiploid sex determining system gives mothers considerable control over the proportion of each offspring sex by selective fertilization of eggs. Moreover, the great diversity in life-history strategies and breeding systems has allowed detailed tests of quantitative predictions linking sex ratios to environmental or genetic factors at the colony and population levels. Nevertheless, the vast majority of theoretical and experimental treatments devoted to sex allocation in ants, bees and wasps have focused on sex ratio at emergence or adult stage, rather than primary sex ratio adjustment (the proportion of each sex at oviposition). Sex ratio at emergence may be adaptive, but it may also result from sex-specific differences in brood mortality during development, whereas primary sex ratio can directly measure the mother's adaptive response to environmental variations and / or socio-genetic conditions. Here, I review current knowledge on primary sex ratio control by queens in social Hymenoptera, especially ants. I present the most classical methods for primary sex ratio determination, and outline empirical studies showing a regulation of the relative number of male and female eggs laid by queens as a function of demographic, ecological and socio-genetic factors. Finally, I propose some directions for future research that should help to clarify the extent of primary sex ratio adjustment in response to environmental conditions, the type of informative cues used by queens to assess their environment, and possible genetic constraints on primary sex ratio adaptation.

Key words: Sex ratio adjustment, haplodiploidy, queen control, demography, population genetic structure, local competition, ecological constraints, ants, social Hymenoptera, review.

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Introduction

Fisher's (1930) central theory of sex ratio predicts that, due to frequency-dependent selection, parents should invest equally in both male and female offspring. If sons and daughters are equally costly, parents will produce the same number of males and females, that is, a numerical sex ratio (proportion of individuals of one sex among offspring) of 0.5. When sons and daughters are not equally costly to produce, the evolutionarily stable strategy (ESS; Maynard Smith & Price 1973) is to invest equally in male and female offspring to obtain an investment sex ratio or sex allocation (proportion of resources allocated to one sex) of 0.5. Frequency-dependent selection also predicts that when a population is skewed toward one sex, the benefits of investing in the other sex increase. The model usually applies to the investment ratio at the adult stage, but it also concerns the sex ratio at birth or, in oviparous species, at the time of egg-laying. Fisher's theory holds: (I) when mating occurs in large, effectively panmictic populations where males and females have equal chances to find a mate, and (II) when the fitness return to the parents per unit of energy expended is equal for the two sexes among offspring. These assumptions are, however, frequently violated under natural conditions. Resource allocation and fitness return may not be identical for male and female offspring because of differential dispersal of sexes, local competition, ecological constraints, parental quality, or mating system. In these circumstances, parents should be selected to bias sex allocation towards the sex with the highest fitness returns (Trivers & Willard 1973, Hardy 1997, 2002, West & Herre 2002, West 2009).

Studies of sex ratio in Hymenoptera (ants, bees, wasps, and sawflies) have provided some of the strongest tests of Fisher's theory, for at least three main reasons. First, the Hymenoptera comprise about 200,000 species with highly variable dispersal strategies that profoundly affect group structure. Second, they show great diversity in life-history strategies, from solitary parasites to highly co-operative societies, with a wide variety of breeding systems. Third, sex determination is based on arrhenotokous haplodiploidy, whereby unfertilized eggs develop into haploid males whereas fertilized eggs become diploid females (Box 1). This system allows females to control the sex of each off-

Box 1: Complementary sex determination under arrhenotokous haplodiploidy.

WHITING (1935, 1943, 1945) first showed that the underlying genetic mechanism of arrhenotokous haplodiploidy is complementary sex determination (csd). Under csd, sex is determined by the complementary composition of alleles at one (single locus, *sl*-csd) or several (multiple loci, *ml*-csd) loci. Heterozygotes at the sex locus (loci) develop into females, while homozygotes and hemizygotes at the sex locus (loci) develop into diploid and haploid males, respectively (COOK 1993, BEYE & al. 2003). The mechanism of csd has an intrinsic genetic load because it results in the production of diploid males. Usually, diploid males suffer from low viability (PETTERS & METTUS 1980), they are sterile (COOK 1993, GODFRAY & COOK 1997) and unable to mate (SMITH & WALLACE 1971), or they produce diploid sperm and sire a triploid, sterile female progeny (NAITO & SUZUKI 1991). In some species, nevertheless, diploid males survive to adulthood and are able to sire fertile diploid female offspring (COWAN & STAHLHUT 2004).

spring via fertilization of the eggs (COOK & CROZIER 1995). Females store the sperm of their mates in their spermatheca and use it to fertilize eggs. By opening or closing the spermathecal valve, mothers can selectively fertilize eggs as they pass through the oviduct, thus controlling the proportion of male and female offspring produced. Parasitoids and fig-pollinating wasps have provided some of the most convincing examples of adaptive sex ratio biases in response to environmental conditions that affect the fitness of sons or daughters differently (KING 1993, GODFRAY 1994, HARDY 1994, ODE & HUNTER 2002, WEST 2009). Surprisingly, however, the vast majority of these studies did not explicitly consider the point at which the adjustment of offspring sex ratio occurs. Most studies measured the sex ratio at emergence. The actual sex ratio produced by females (i.e., the proportion of each sex laid) was usually only inferred based on variation in sex proportions at the adult stage (CHARNOV & al. 1981, WRENSCH & EBBERT 1993, GODFRAY 1994, WEST & HERRE 2002, WEST & SHELDON 2002). The sex ratio at emergence may, however, partially result from factors such as sex-specific differences in brood mortality during development due to the presence of lethal recessive alleles that are fully expressed in haploid males (SMITH & SHAW 1980), to differential tolerance for resource competition (KING 1993, NAGELKERKE & HARDY 1994), to sex specific predation or parasitism (PEREIRA & DO PRADO 2005), or to differential allocation of parental care to male and female brood (TRIVERS & HARE 1976). Sex-specific mortality is probably not consistent across breeding seasons, and no study has shown that females can bias the proportion of each sex laid to compensate for possible differences in male and female brood mortality according to environmental constraints. Therefore, assessing the sex ratio at the egg stage could more directly measure a female's response to environmental conditions via sex ratio skews in her offspring.

Social Hymenoptera add a further level of complexity in sex ratio studies, because their organization is rooted in reproductive division of labor based on a system of caste differentiation. Queens and males are responsible for reproducing, whereas workers usually forego their own direct reproduction and perform other colony-maintenance tasks (foraging, defending the nest, caring for the brood). This system has two important consequences. First, queens should be selected to adjust the proportion of male and female eggs laid in response to two issues: the need for workers (versus reproductives) to ensure colony growth and efficiency (Fig. 1), on the one hand, and the sex ratio of reproductive offspring, on the other hand. Second, colony sex ratio is potentially under the control of two parties: the queens laying the eggs and the workers rearing the brood to maturity. This dual control over sex ratio is critical because queens and workers may have different reproductive optima (HAMILTON 1972, TRIVERS & HARE 1976). The male-haploid sex determining system indeed results in asymmetries of relatedness within colonies. Males being haploid, their germinal cells do not undergo meiosis and all spermatozoids of a given father are genetically identical (except for mutations). Therefore, daughters inherit the same genes from their father and they share, on average, half the genetic contribution of their mother. On the other hand, the genetic makeup of males is entirely derived from the mother. As a consequence, workers are generally more closely related to sisters than to brothers. Workers, who reproduce indirectly by rearing the queen's offspring, can therefore maximize their inclusive fitness by favoring female-biased broods. By contrast, queens are equally related to offspring of both sexes and are selected to favor an even sex investment ratio. Note that sex ratio in social Hymenoptera usually refers to the new reproductives (the new queens and males) produced in a colony, and not the new cohorts of non-reproductive workers. Considerable theoretical and empirical attention has been paid to the influence of within-colony relatedness asymmetries over sex allocation in social Hymenoptera. The prediction of a female-biased sex investment ratio was found by several authors based on different theoretical models (TRIVERS & HARE 1976, CHARNOV 1978, BULMER & TAYLOR 1981, PAMILO 1991). Theory was then extended for situations in which some basic assumptions do not hold (e.g., multiple mating by queens, multiple queens per colony, worker reproduction) and for species with unusual life-history strategies (e.g., social parasites) (reviewed in BOURKE & FRANKS 1995, CROZIER & PAMILO 1996). As for solitary species, the vast majority of theoretical and experimental treatments devoted to sex allocation in ants, bees and wasps focused on the sex ratio at the adult stage, and were conducted at the colony and / or population level. Most studies did not disentangle the respective contribution of the queen and the workers in colony sex ratio determination. Some theoretical works highlighted that queens should have considerable control over the colony investment ratio by adjusting the number of haploid and diploid eggs laid, thereby forcing workers to operate within this constraint (BULMER 1981, BULMER & TAYLOR 1981, PAMILO 1992). Nevertheless, the degree of control that mothers have over the number and sex of each offspring laid remained surprisingly poorly studied experimentally. This stems (I) from the difficulty in determining the sex of the eggs laid (haploid *vs.* diploid), and (II) from the fact that it is usually impossible to determine whether

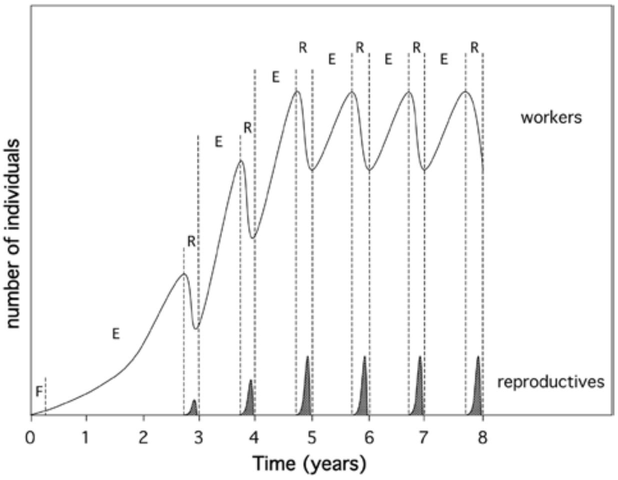

Fig. 1: Demography of perennial colonies of most ant species. Colony life cycle is typically divided into a founding stage (F), an ergonomic stage (E) and a mature, reproductive stage (R). In temperate climates, the rearing of reproductive brood corresponds to a reduction in worker production; after the release of the reproductive forms during the breeding season, colonies return to an ergonomic stage by rearing only workers to ensure colony growth. Modified from Oster & Wilson (1978) and Tschinkel (1983).

diploid eggs will develop into queens or workers, so that egg sex ratio determination does not allow disentangling between sex allocation (male vs. new queens) and resource allocation (sexual vs. workers) when all three castes are produced at the same time of the year.

The aim of the present review is to summarize current knowledge on primary sex ratio control by queens in ants and other social Hymenoptera. I consider the primary sex ratio as the numerical proportion of haploid eggs among all the eggs (haploid and diploid) at the time of laying, and the secondary sex ratio as the numerical proportion of males among reproductive offspring (males and new queens) at maturity. First, I will briefly describe the methods used for primary sex ratio determination. Second, I will present demographic and ecological factors, as well as the effects of local population genetic structure likely to influence the primary sex ratio, and summarize empirical studies showing a regulation of the relative number of male and female eggs laid by queens as a function of these parameters. Third, I will outline the relative influence of queens and workers on reproductive decisions of the colony, with particular focus on the ability of queens to influence colony secondary sex ratio by adjusting the primary sex ratio. Finally, I propose some avenues about the direction of future research.

Primary sex ratio determination in social Hymenoptera

Despite its theoretical interest, few studies aimed at testing primary sex ratio control by queens in social Hymenoptera. This was largely due to the difficulty in estimating the proportion of haploid and diploid eggs laid. The problems of sexing eggs with confidence have now been largely solved thanks to the development of molecular techniques allowing differentiation between haploid-males and diploid-females at early stages of development. Below, I give a brief overview of the most common methods used for primary sex ratio determination in social Hymenoptera. Because the exact genetic basis of sex determination is only currently known for the honeybee *Apis mellifera* (sex is determined by a single multi-allelic locus, the complementary sex determiner (csd) locus; Beye & al. 2003), all the methods used to date for sexing the eggs in the Formicidae are based on determination of the ploidy level.

Karyotypes: Data on primary sex ratio were first obtained from karyotype studies (Imai & al. 1977, Hasegawa 1992, Aron & al. 1994). Chromosomes are stained with various dyes and their number in each nucleus is counted under a light microscope. Nuclei from male eggs contain half the number of chromosomes than nuclei from female eggs. The method is cheap, easy and requires no technical skills, but can be tedious and time-consuming. The optimal "age" of eggs (the time since they were laid) must be determined because younger eggs may not have enough cells in metaphase, while older ones may have too many. Sex determination is successful for 50 - 60% of the eggs, so that reliable estimates of the primary sex ratio require relatively large samples.

Fluorescence in situ hybridization (FISH): This method involves hybridizing a specific DNA probe, labeled with a fluorochrome, with its complementary target DNA sequence (Muleris & al. 1996). The difficult part of the technique is to obtain a DNA-probe from a genomic DNA library; moreover, the probe is not expected to work across species. These problems have been solved for most ant species. de Menten & al. (2003) made a probe from a coding sequence of 4.5 kbp of the *abdominal-A* gene known to specify the identity of the most abdominal segments in insects (Hughes & Kaufman 2002). The coding sequence presents a high level of conservation among the different subfamilies of ants (Niculita & al. 2001, de Menten & al. 2003). Egg ploidy level is uncovered on the basis of the number of spots observed in nuclei under epifluorescence microscope. Haploid eggs reveal a single fluorescent spot / nucleus, diploid eggs two spots / nucleus, and eventually polyploid cells > 2 fluorescent spots / nucleus. The probe was shown to reliably determine the sex (haploid or diploid) of eggs in ten species belonging to eight subfamilies of Formicidae. However, FISH requires tedious, sustained lab work and complex histochemistry manipulations, including tagging of a cDNA or a cRNA probe, fixation of cells, hybridization of the probe to the target sequence at high temperature, antibodies treatment and staining.

Microsatellite DNA markers: Genetic sexing based on microsatellite marker loci has been revealed to be highly accurate and it has been heavily exploited to differentiate diploid heterozygotes from haploid hemizygotes among eggs in ants, bees and wasps (Arevalo & al. 1998, Ratnieks & Keller 1998, Passera & al. 2001). Isolation and characterization of microsatellite markers has, in recent years, become quite affordable, and genotyping of microsatellite markers is now a routine process in most laboratories. Egg sex determination may, however, be hindered when genetic variation is reduced, which may occur when populations have been through bottlenecks or in species with a high level of inbreeding. In the latter situation, homozygous diploids are frequent and undistinguishable from haploid hemizygous.

Flow cytometry (FCM): Recently, flow cytometry has emerged as a fast, inexpensive and reliable alternative to discriminate between male and female brood in haplodiploid organisms (Aron & al. 2003, Aron & al. 2004). This cytogenetic approach allows determination of ploidy level based on measurement of nuclear-DNA content (Boeck 2001, Kron & al. 2007). Nuclei from an individual (egg, larva, adult) are stained with a DNA-specific fluorochrome that intercalates with the chromosomes in direct proportion to the amount of the DNA. Then, they are illuminated by a mercury lamp or one to several laser beams; the fluorescence emitted from each stained nucleus is registered and quantified. FCM is a high throughput technique because it allows analyzing hundreds of cells within seconds. The only limitation of FCM for primary sex ratio determination is that users must avoid sampling very freshly laid eggs. Indeed, cell cycle in young embryos tends to be synchronous, so that a majority of cells concurrently experience the same stage of the cycle. If sampling occurs when most nuclei are in the stage G2 / M of the cell cycle, a haploid embryo may be erroneously considered as a diploid one. Working on eggs a few days old is therefore recommended.

Morphology and / or histology: Morphological and histological approaches were also used for early sex brood determination in some species. However, unlike the methods above, they cannot be employed for sexing eggs but only larval stages. They focus on larval cuticular structures, internal anatomy or both. Sex of first instar larvae can be distinguished on the basis of cuticular and sub-cuticular gonadal structures in bumble bees (Duchateau & Van Leewen 1990), and on differences in the size and the shape of the epiproct in the honeybee (Santomauro & Engels 2002). In ants, accurate determination of male and female individuals from the first larval instar was reported in some species, based on sex specific differences in the form, size and histological organization of reproductive organs (Ortius-Lechner & al. 2003). Differences between larval sexes at later stages of development (i.e., 3rd instar and the following ones) were also documented, based on the size, color and cuticular structure in ants (Brian 1981, Berndt & Kremer 1986, Edwards 1991, Passera & al. 1995), and on gonadal morphology and gonopore structure in wasps (Cotoneschi & al. 2007). A major advantage of morphological methods is that they allow correct assignation of diploid males as males rather than diploid females. However, because diploid males arise from fertilized eggs, they are "intended" to be females and can reasonably be entered as such in determining the primary sex ratio established by selection.

Influence of ecology, demography and competitive interactions between relatives on primary sex ratio determination

A number of distinct ecological, demographic and population genetic contexts can markedly affect offspring fitness and have been shown to influence the proportion of haploid and diploid eggs laid by queens in social Hymenoptera. They include seasonal variations in sexual production, the colony life-history stage, as well as population genetic structure and local competition among related offspring.

Seasonal variations: Colonies of most ant species show a K-type life-history strategy, with a low reproductive rate, long life expectancy, high population stability, extensive parental care and repeated reproduction (Oster & Wilson 1978, Hölldobler & Wilson 1990). Colony demography is closely associated with the life cycle, which is typically divided into three stages: a founding stage, an ergonomic stage and a mature, reproductive stage (Fig. 1). The two first stages of the cycle are devoted to mating, finding a suitable nest site, and worker production to ensure colony growth. After a period ranging from a few months to several years depending on species, colonies enter the reproductive stage and start producing reproductive forms. At the adult stage, the demography of the perennial colonies follows a seasonal cycle related to its needs throughout the year. In temperate climates, the rearing of reproductive brood begins in early / mid-spring and culminates in summer with the release of the sexual adults (males and virgin queens) during the breeding season. Then, colonies return to an ergonomic stage by rearing only workers to ensure colony growth.

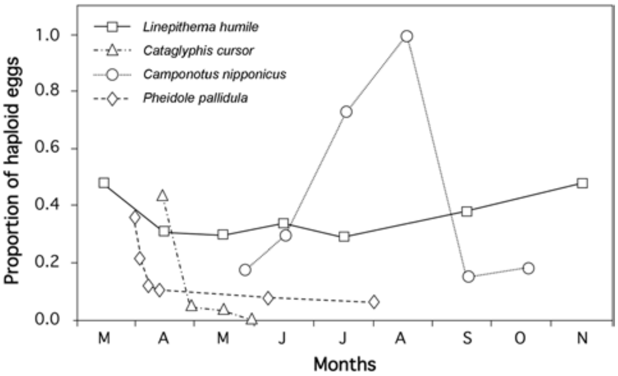

Fig. 2: Changes over time in the primary sex ratio laid by queens. Data for *Linepithema humile* and *Camponotus nipponicus* obtained from eggs collected in the field throughout the period of egg-laying. Data for *Pheidole pallidula* and *Cataglyphis cursor* obtained after overwintering under laboratory conditions; under natural conditions, queens start egg production around the end of March and mid-April, respectively. Derived from Hasegawa (1992), Keller & al. (1996a) and Aron & al. (1994, 2011).

Several studies have shown that ant queens modify the ratio of male to female eggs laid depending on the season (Fig. 2). In the nocturnal and arboreal Japanese ant *Camponotus nipponicus*, queens lay eggs from May to October. However, haploid egg production greatly varies with more than 80% of eggs being male-destined in July and August, whereas this proportion falls to 20% in the rest of the year (Hasegawa 1992). All the eggs laid during the year develop into larvae by November. Though adult workers may possibly emerge from eggs laid early in the year, the vast majority of small larvae arise from eggs laid in July - September, which overwinter and develop into pupae the following year. A significant seasonal variation in the primary sex ratio has also been shown in introduced populations of the Argentine ant *Linepithema humile* (formerly *Iridomyrmex humilis*) in France (Aron & al. 1994). Whereas the proportion of haploid eggs laid by the queens is close to 50% at the end of the winter, it decreases to a value close to 30% in spring and summer. Afterwards, the proportion of haploid eggs increases again reaching a value close to 50% at the onset of hibernation. These changes in the primary sex ratio are closely correlated with the monthly mean temperature. In the Argentine ant, males come from the overwintering brood and from the first eggs laid in early spring, that is, when the proportion of haploid eggs laid is close to 50%. Changes in the proportion of haploid eggs laid by queens as a function of the time were also documented in *Pheidole pallidula* (KELLER & al. 1996a) and *Cataglyphis cursor* (ARON & al. 2011). In both these species, haploid eggs are laid in early spring, when workers rear the brood into sexuals. Outside this period, queens lay few or no haploid eggs. Altogether, these observations suggest that ant queens adaptively modify offspring sex ratio over time, by increasing the proportion of haploid eggs laid during periods of sexual production.

The ability of queens to regulate the proportion of haploid and diploid eggs laid over time was also reported in the honeybee *Apis mellifera*, where the ratio of drone eggs to worker eggs is larger during the swarming season than in other periods of the year (SASAKI & al. 1996). Interestingly, in the honeybee the ratio of drone and worker eggs laid depends on queen oviposition history and results, at least in part, from a negative feedback process of drone egg production (SASAKI & al. 1996, WHARTON & al. 2007). Queens prevented from laying drone eggs for a short period of time (by giving them experimental combs with worker cells only) later compensated by producing a more male-biased egg sex ratio than queens who had not been prevented from laying drone eggs. As a result, both prevented and non-prevented queens produced approximately the same number of male eggs in total. Thus, each queen compensates for her own production of drone eggs based on the amount of male eggs laid previously. This supports the notion that queens regulate the total number of drone eggs they lay and that they attempt to achieve a given ratio of drone eggs to worker eggs that is appropriate for the time of the year. This interpretation is consistent with the fact that honeybee queens sometimes lay eggs in a drone to worker ratio that deviates from the ratio of available drone to worker cells, indicating that they control the sex of their offspring by selecting what type of cell to use.

Mode of colony foundation and life-history stage: In the life of ant colonies, founding is probably the most vulnerable stage because of predation, strong competition among incipient colonies, nest usurpation or low resistance to adverse climatic conditions (HÖLLDOBLER & WILSON 1990, TSCHINKEL 1992a, b, HERBERS 1993). In several species, colonies are initiated by independent foundation (ICF), that is, without the help of a worker force. Newly mated queens seal themselves into a chamber and rear their first brood solely (haplometrosis) or in association (pleometrosis) from stored fat and histolysis of their wing muscles. Alternatively, colony reproduction proceeds by dependent foundation (DCF), a process whereby young mated queens and workers walk away from their natal colony to establish a new nest in the vicinity. These two strategies result in different selection pressures on young queens (PEETERS & MOLET 2009). In species with ICF, queens are under strong selection to produce a large worker force as quickly as possible, before depletion of their own body reserves and because they may suffer high mortality owing to competition during the founding stage when few or no workers can defend the colony. In addition, colonies have first to pass through an ergonomic stage before they can produce the first reproductive offspring. In contrast, new colonies initiated by DCF start with a worker force, which results in a lower mortality rate and allows earlier production of reproductive offspring. As a consequence, one would predict that, in species founding independently, queens of incipient colonies should produce mostly worker brood by laying a lower fraction of haploid eggs than would queens in mature colonies. Such a difference in the ratio of haploid and diploid eggs laid with the stage of colony development is not expected in most species founding dependently.

Consistent with this prediction, primary sex ratio analyses show that in the garden ant *Lasius niger*, where new colonies are initiated by ICF, queens of incipient colonies lay a lower proportion of haploid eggs than queens from mature colonies (0.05 vs. 0.24). By contrast, in the Argentine ant *Linepithema humile* where DCF is the rule, young freshly mated queens lay a similar proportion of haploid eggs to older queens from mature colonies (0.17 vs. 0.14) (ARON & PASSERA 1999). Thus, queens seem to adjust the primary sex ratio according to the mode of foundation and the life-history stage of the colonies. These two species, however, belong to different subfamilies within the Formicidae (Formicinae and Dolichoderinae, respectively), and given that phylogenetic constraints might also somewhat influence egg-laying patterns, future studies should aim to compare the primary sex ratio within species where both strategies of colony founding coexist (HEINZE & KELLER 2000).

Local population genetic structure and competition among relatives: When dispersal distance varies between the sexes, relatives of the less-dispersing sex are more likely to interact with one another. Such interactions can result in competition among related individuals for access to resources. In this situation, the fitness return from investing into the less-dispersing sex is a diminishing function of the total investment in sexuals, i.e., the less-dispersing sex becomes devaluated. Parents would therefore benefit from producing the sex that will experience minimum levels of competition. A detailed description of variations in sex allocation patterns with investment in sexual offspring under local competition is given by FRANK (1987, 1990).

Local mate competition (LMC): Under LMC, relatives – usually males – compete for access to the opposite sex (HAMILTON 1967). When brothers compete among themselves for a limited number of mates, the reproductive value of sons decreases as investment in sexual offspring increases. The more numerous males are, the more severely they will engage in competition with other males bearing the same genes, leading to an increased proportion of unproductive sons. Thus, parental fitness will increase if more resources are allocated to daughters; the female bias reduces competition among brothers for mates and increases the number of mates per son.

Competition among related males for access to females in a locally restricted area is considered to be fairly rare in ants. In most species, mating takes place during population-wide nuptial flights in which numerous colonies synchronously release male and female sexuals, thereby greatly decreasing the probability of close relatives experiencing mate competition (CROZIER 1980). LMC has been documented in a few species where mating occurs near to or in the nest due to males being flightless and / or females dispersing farther than males: e.g., the two inquiline parasites *Myrmoxenus* (*Epimyrma*) *kraussei* (WINTER & BUSCHINGER 1983) and *Plagiolepis xene* (ARON & al. 1999b), and the free-living species *Messor aciculatus* (HASEGAWA & YAMAGUCHI 1995), *Technomyrmex albipes* (TSUJI & YAMAUCHI 1994), *Myrmica sulcinodis* (PEDERSEN & BOOMSMA 1998), *Hypoponera opacior* (FOITZIK & al. 2010), as well as several representatives belonging to the genus *Cardiocondyla*, namely *C. wroughtoni* (KINOMURA & YAMAUCHI 1987), *C. obscurior* (CREMER & HEINZE 2002), *C. batesii* (SCHREMPF & al. 2005), *C. kagutsuchi* (YAMAUCHI & al. 2005) and *C. minutior* (SUEFUJI & al. 2008). As expected from LMC theory, the sex ratio (both numerical and investment) among adult sexuals is highly female-biased. However, whether this female-bias results from queens biasing the proportion of each sex at laying, or from manipulation of the sex ratio by the workers during brood rearing (or from both), remains largely unstudied.

Under LMC, both the queens and the workers should favor a female-bias among sexuals. The primary sex ratio laid by the queens at the period of sexual production is therefore predicted to be female-biased. A first test of the hypothesis comes from the comparison between the primary and the secondary sex ratio in the inquiline parasite ant *Plagiolepis xene* (ARON & al. 1999b). Inquiline parasites have lost the worker caste and exploit the resources and workers of a host species (here, *P. pygmaea*) to raise their brood. Queens of the parasite produce only sexuals, which are reared by the host workers. The latter are unrelated to the brood of the parasite and, hence, have no evolutionary stake in biasing sex allocation. Consistent with LMC, both the primary sex ratio (0.13) and the secondary sex ratio (0.14) are highly female-biased in the inquiline species. Moreover, sex ratio values do not differ from each other, indicating that the female-biased sex ratio is achieved by queens laying a higher proportion of diploid eggs rather than by a higher mortality of haploid males during development.

In a more thorough study, DE MENTEN & al. (2005a) showed that queens of the ant *Cardiocondyla obscurior* adjust the proportion of haploid eggs laid in response to different intensities of local competition. All species of the genus *Cardiocondyla* are characterized by a peculiar male polymorphism, with winged males specialized in dispersal and wingless, ergatoid "fighter" males mating inside the nest (HEINZE & al. 2005). Ergatoid males do not leave the nest; they engage in lethal combats with rivals to mate with the virgin queens produced in their natal colony. In *C. obscurior*, colonies may be headed by a variable number of queens due to adoption of new reproductive females, or merging or splitting of nests. Variation in queen number between colonies results in differences in the strength of LMC, since relatedness between competing males changes with the number of nestmate queens. In monogynous colonies, a single queen produces all males and relatedness between competing males is high (they are full brothers). In polygynous colonies, several queens produce male offspring, so that nestmate males are less related to each other and, hence, LMC should be relaxed. In line with LMC theory, colony-level secondary sex ratio varies in response to queen number (CREMER & HEINZE 2002). Single-queen (monogynous) colonies produce fewer competing, ergatoid males and a more female-biased sex ratio than multiple-queen (polygynous) colonies. In contrast, there is no difference in the proportion of dispersing, winged males produced between both colony types. In fact, variation in the secondary sex ratio between both colony types results from an adaptive adjustment of the primary sex ratio laid by queens in response to the intensity of LMC (DE MENTEN & al. 2005a). Queens of *C. obscurior* lay a significantly lower proportion of haploid eggs in single-queen colonies than in multiple-queen colonies (0.08 and 0.18, respectively). Remarkably, experimental changes in colony queen number induce an adjustment of the primary sex ratio: Within six weeks, queens reduce the proportion of haploid eggs laid when the relatedness among the competing males increases in the colony.

Local resource competition (LRC): When dispersal of females is limited, related females may compete for access to local resources (nest sites, food). In this situation, the reproductive value of daughters decreases with increased investment in sexual offspring, since the more numerous females are, the more they will experience competition (CLARK 1978, FRANK 1987). LRC predicts an association between productivity and sex ratio, with more productive parents investing proportionally more in male production, and an overall male-biased sex allocation.

In ants, harsh ecological constraints such as nest-site limitation, predation, or competition between colonies are known to restrict female dispersal and favor LRC in two complementary ways (HERBERS 1993, FOITZIK & HEINZE 1998, PEDERSEN & BOOMSMA 1999). First, newly inseminated queens may return to their natal nest after the nuptial flight or seek adoption into neighboring colonies, thereby increasing colony queen number. Second, high risks associated with solitary foundation may promote dependent foundation (DCF), whereby new colonies are initiated in the vicinity of the mother nest. DCF occurs in both monogynous and polygynous species (PEETERS & MOLET 2009). It results in a strong population genetic structure, and is usually associated with a male-biased population-wide sex ratio. LRC accounts for a male-bias among the sexuals produced in the monogynous ants *Protomognathus americanus* (FOITZIK & HERBERS 2001) and *Cataglyphis cursor* (PEARCY & ARON 2006). It also contributes to the male-biased sex ratio reported in several polygynous species such as *Rhytidoponera impressa* – type B (WARD 1983), *Leptothorax acervorum* (CHAN & BOURKE 1994, CHAN & al. 1999), *Myrmica sulcinodis* (PEDERSEN & BOOMSMA 1998), *M. ruginodis* (WALIN & SEPPÄ 2001), *Hypoponera opacior* (FOITZIK & al. 2010), as well as several species of the genus *Formica* (PAMILO & ROSENGREN 1983, SUNDSTRÖM 1993, 1995).

BROWN & KELLER (2000) stressed that colony queen number should directly affect the intensity of LRC in polygynous species. They proposed the queen-replenishment hypothesis to account for variations in sex investment ratio across colonies. Their hypothesis predicts that colonies containing many queens (i.e., experiencing high LRC) should produce a male-biased investment ratio, whereas colonies hosting few queens (i.e., experiencing reduced or no LRC) should produce a female-biased ratio. Consistent with this prediction, in the ant *Formica exsecta* highly polygynous colonies produce a more male-biased secondary sex ratio than weakly polygynous colonies (BROWN & KELLER 2000, 2002). Additionally, experimental manipulation of queen number over three consecutive years led to variations in the sex ratio produced by colonies in the expected direction: Queen removal resulted in a significant increase in the proportion of colonies producing new queens (KÜMMERLI & al. 2005).

In a kind of mirror image of the LMC, under LRC both the queens and the workers are selected to favor a male bias among sexuals. To the best of my knowledge, no study has been dedicated to the adjustment of the proportion of male eggs in response to the strength of LRC or as a function of the number of reproductive females in a colony, in ants and other social Hymenoptera. Yet, recent works suggest that queens could bias the primary sex ratio in response to the effect of LRC in the monogynous ant *Cataglyphis cursor* (ARON & al. 2011). LRC is a primary factor shaping sex allocation in this species: It is male-biased at both the colony and population levels, and the investment in males, but not in females, is positively correlated with total investment in sexuals (PEARCY & ARON 2006). A remarkable feature of the species is that workers arise from fertilized eggs, while new queens are produced asexually through thelytokous parthenogenesis (PEARCY & al. 2004). Males arise from arrhenotokous parthenogenesis, as is usually the case in Hymenoptera. Since both male and new queens are produced from unfertilized eggs, the primary sex ratio laid by the queens at the period of sexual production (early spring) basically corresponds to the proportion of arrhenotokous eggs among parthenogenetic ones (arrhenotokous and thelytokous). Consistent with the LRC theory, the primary ratio produced by queens when they resume egg-laying after hibernation is significantly male-biased, on average 0.69; this value rapidly decreases and no haploid eggs are laid after a few weeks (ARON & al. 2011). The proportion of arrhenotokous eggs laid among parthenogenetic ones (0.69) does not differ from the numerical proportion of males reared among adult sexuals (0.71) (S. Aron, unpubl.). Thus, queens bias the primary sex ratio towards males at the period of sexual production, possibly to optimize offspring fitness, and workers do not or only weakly alter the proportion of each sex during brood development.

Local resource enhancement (LRE): Biased sex ratios may also occur when relatives co-operate to enhance their reproductive success. In this situation, selection favors overproduction of the most philopatric sex. Local resource enhancement (TAYLOR 1981) consistently explains the female-biased secondary ratio in several bee species, including the primitively social allodapine *Exoneura bicolor*. Nestmate females are closely related, and both per capita reproduction and colony survivorship increase (up to a certain threshold) with group size (SCHWARZ 1987, 1988). Nevertheless, the numerical proportion of males produced in a nest is positively correlated with the number of offspring reared: More females are reared in small broods and, as brood size increases, more males are produced. This fits well with the theory, as the benefits of producing extra females that will cooperate and nest decreases with the number of females in a group. Whether sex ratio variations result from females adjusting the primary sex ratio or from differential rearing of both sexes according to brood size remains unknown.

Social conflicts, cooperation and primary sex ratio determination

Queen-worker conflict and cooperation over sex ratio: In social Hymenoptera, the haplodiploid sex determining system results in relatedness coefficients that are not uniform among colony members. Assuming no inbreeding, in a colony headed by a single, once-mated queen, workers are three times more related to their sisters ($r = 0.75$) than to their brothers ($r = 0.25$), and should be selected to drive a 3:1 female:male sexual investment ratio. By contrast, because queens are equally related to their sons and daughters ($r = 0.5$), natural selection should act on queens to favor an equal investment (1:1 male:female) in both sexes. Several factors may, however, greatly affect colony kin structure and the potential for conflicts over sex allocation in social Hymenoptera (HAMILTON 1972, RATNIEKS 1988, BOOMSMA 1993, BOOMSMA & RATNIEKS 1996, RATNIEKS & al. 2006). Two primary factors of the variation in social structure are (I) the occurrence of multiple reproductive queens in a colony (polygyny) and (II) multiple mating by queens (polyandry). When the number of related queens in a colony is elevated or when queens mate with a high number of males, the mean relatedness among workers is reduced and they are on average equally related to the males and female sexuals produced in the colony. As a result, the interest of the queen and the workers converge and both parties may enhance their inclusive fitness by favoring an equal investment in the sexes. In this case, the queen and the workers should co-operate to adaptively regulate the colony investment in reproduction (WHARTON & al. 2007, 2008).

Worker control over sex ratio: Because workers rear the brood and outnumber the queen(s), and because their ability to enhance inclusive fitness by biasing colony sex investment ratio is an integral part of hypothesis on the evolution of sociality in Hymenoptera (HAMILTON 1964, TRIVERS & HARE 1976), workers are expected to have at least partial control over sex ratio and to achieve their preferred optimum set by relatedness asymmetry (RA). Consistent with this prediction, an association between sex allocation and patterns of relatedness within colonies has been shown in a number of species (BOURKE & FRANKS 1995, CROZIER & PAMILO 1996, CHAPUISAT & KELLER 1999). The general concept of worker control over sex allocation was firmly-rooted from tests of the relative relatedness asymmetry hypothesis (BOOMSMA & GRAFEN 1990), which was proposed to account for strong sex ratio specialization. The theoretical background of the hypothesis is that kin structure may greatly vary between colonies within populations, due to differences in the number of queens, their relatedness, queen-mating frequency or the extent of worker reproduction. BOOMSMA & GRAFEN's (1990) prediction holds that, at the colony level, workers should benefit by rearing the sex to which they are relatively more closely related as compared to the population average. Under worker control, colonies with a RA above the population average should specialize in the production of females, whereas colonies with a RA below the population average should be mostly male-producing. On the contrary, under queen control, sex ratios should not be "split" because queens are equally related to both offspring sexes. In line with BOOMSMA & GRAFEN's (1990) hypothesis, empirical studies have demonstrated that sex allocation correlates with colony relatedness asymmetry as predicted under worker control in several bee, wasp, and ant species (reviewed in BOURKE & FRANKS 1995, CROZIER & PAMILO 1996, QUELLER & STRASSMANN 1998, CHAPUISAT & KELLER 1999, FOSTER & RATNIEKS 2001, MEHDIABADI & al. 2003; see also SUNDSTRÖM & BOOMSMA 2000 for the consequences of the sequential use of sperm from each mate of the queen on variations in within-colony RA and sex ratio). Across all studies on split sex ratio in social insects, variation in RA explains on average 28% of the variance in sex allocation (MEUNIER & al. 2008).

Comparison between the primary and the secondary sex ratio has shown that ant workers bias the secondary sex ratio of reproductives in two ways.

(1) In several species, the secondary sex ratio (i.e., at the adult stage) is less male-biased than the egg sex ratio, indicating that workers selectively eliminate male brood during development (*Linepithema humile*: ARON & al. 1994, PASSERA & ARON 1996; *Solenopsis invicta*: ARON & al. 1995; *Pheidole pallidula*: KELLER & al. 1996a; *Formica exsecta*: SUNDSTRÖM & al. 1996, CHAPUISAT & al. 1997; *F. selysi*: ROSSET & CHAPUISAT 2006; *Plagiolepis pygmaea*: ARON & al. 1999b, 2004). The difference in the proportion of males between the egg and adult stages when queen-worker conflicts over sex allocation are expected on the basis of relatedness values, but not when genetic conflicts are absent, shows that male brood reduction stems from workers manipulating the sex ratio rather than from differential mortality of males during development. Interestingly, male brood elimination by workers may also stem from co-operation between the queen and the workers if it permits tailoring of reproductive efforts of the colony to resource availability or other environmental conditions (CHAPUISAT & al. 1997, WHARTON & al. 2008). In the honeybee *Apis mellifera*, queen-worker conflict over sex ratio is expected to be minimal because queens are highly polyandrous (PALMER & OLDROYD 2000, TARPY & al. 2010). Yet honeybee workers conditionally eliminate male larvae. Experimental manipulation of the abundance of male brood shows that the survival of newly produced drone brood is higher in colonies with an absence of older drone brood than in colonies with an excess of older drone brood (WHARTON & al. 2008). This indicates that workers cull male larvae to adaptively adjust male reproductive function, possibly to enhance overall colony efficiency and success.

(2) The second mechanism by which workers may bias sex allocation is by controlling the caste fate of female brood, i.e., whether a diploid larva will develop into a new reproductive queen or a sterile worker. Here, the proportion of males is the same at the egg and adult stages, but workers attain a more female-biased sex allocation by raising a greater proportion of diploid brood as sexuals, most likely through differential feeding of developing larvae. Such worker control of the female caste fate has been documented in the ants *Leptothorax acervorum* (HAMMOND & al. 2002) and *Aphaenogaster smythiesii japonica* (IWANISHI & al. 2007).

Queen control over sex ratio: Although in many species workers have great power over colony sex ratio determination, worker control is not a rule. Sex allocation was indeed shown unlinked to between-colony variation in relatedness asymmetry in various species, including the bumblebee *Bombus hypnorum* (BROWN & al. 2003) and several ants like *Formica sanguinea* (PAMILO & SEPPÄ 1994), the polygynous form of *Formica exsecta* (BROWN & KELLER 2000), *Pheidole desertorum* (HELMS 1999), *Solenopsis invicta* (VARGO 1996), *Leptothorax nylanderi* (FOITZIK & HEINZE 2000), *Myrmecina nipponica* (MURAKAMI & al. 2000), *Lasius niger* (FJERDINGSTAD & al. 2002, JEMIELITY & KELLER 2003), or *Pheidole pallidula* (FOURNIER & al. 2003). Workers may lack information to manipulate sex ratio towards their own reproductive interest, or sex ratio manipulation may be too costly for workers (REUTER & KELLER 2001, REUTER & al. 2004, HELMS & al. 2005, HELANTERÄ & RATNIEKS 2009). However, several studies revealed that sex allocation bias also have other evolutionary causes, such as queen control or competition among co-breeding queens (KÜMMERLI & KELLER 2009).

In theory, queens can bias colony sex allocation in three ways that are not mutually exclusive (REUTER & KELLER 2001, ROISIN & ARON 2003). First, queens may vary the temporal availability of haploid and diploid eggs laid, for instance by laying a male-biased primary ratio at the time of sexual production to force workers rear more males. Second, queens may limit the amount of eggs laid in the colony. In species that produce males, new queens and workers at the same time, workers cannot adaptively bias sex allocation unless eggs produced by the queen are in excess of those that can be reared as reproductives with available resources. If queens limit the number of diploid eggs available, workers should raise all diploid eggs as workers and release only male sexuals. Third, queens may regulate the caste ratio among diploid brood at the period of sexual production, by hormonally determining their development into the queen or the worker caste.

(I) Primary sex ratio control by queens: Queen-worker conflict over sex ratio is expected particularly exacerbated in populations with high RA or when RA vary between colonies. In the monogynous form of the fire ant *Solenopsis invicta,* all colonies consist of one singly mated queen and her completely sterile daughter workers. This family structure leads to maximal RA (3:1 female:male) in all colonies, and workers should favor a three times more female-biased investment ratio than queens. Workers of the fire ant are indeed able to recognize and selectively eliminate males (ARON & al. 1995). Yet, population sex investment ratio lies between the queen and worker optima (1.5:1 female bias) (VARGO 1996). Monogynous populations of the species are characterized by a strong split sex ratio, with some colonies almost exclusively producing males and others female sexuals. Cross-fostering experiments, whereby queens were swapped between male- and female-specialist colonies, quickly reversed the numerical sex ratio: male-specialist colonies that were given a queen from a female-specialist colony switched to producing mostly female sexuals, whereas all female-specialist colonies that received a queen from a male-producing colony switched to producing males (PASSERA & al. 2001). No such changes in the secondary sex ratio occurred when colonies were given a foreign queen from the same colony type. Primary sex ratio analyses showed that queens from male-producing colonies lay more than 50% haploid eggs, whereas queens from female-producing colonies produce almost no haploid eggs. Thus, queens of the fire ant exert partial control over colony sex ratio by enhancing male production through limiting the number of diploid (female) eggs produced.

In the wood ant *Formica selysi*, colonies are headed by one singly mated queen, a doubly mated queen, or several queens. This results in large variations between colonies in RA values. Populations of the species show a highly pronounced split sex ratio, with colonies investing more than 90% of the energy to one sex. According to the RA hypothesis, such a bimodal sex ratio should stem from workers favoring production of females in colonies with relatively high RA compared to the population average, and production of males in colonies with relatively low RA. Rather, sex-ratio specialization in *F. selysi* was shown to stem from queens biasing the sex ratio of their eggs (ROSSET & CHAPUISAT 2006). Primary sex ratio analyses indeed show that queens from male-specialist colonies lay almost exclusively haploid eggs, while queens from female-specialist colonies lay mostly diploid eggs (average proportion haploid eggs laid: 0.99 and 0.09, respectively). Queen control is clearly demonstrated by the strong correlation, close to 0.99, between primary and secondary sex ratios. The bias in egg sex ratio constrains workers' ability to manipulate colony sex allocation by forcing them to rear females in some colonies and males in others. However, as in the fire ant, the change in the proportion of males between the egg and pupae stages suggests that workers eliminate a part of the male brood in some colonies. Indeed, the population sex-investment ratio is slightly female-biased (1.36:1).

Thus, split sex ratio in both species occurs because queens produce a haploid- or diploid-biased primary sex ratio, thereby forcing workers to rear mainly males or new queens.

(II) Primary sex ratio bias and control of caste ratio by queens: Sex ratio specialization of reproductive offspring also occurs in several species of the ant genus *Pheidole* (ARON & al. 1999a, HELMS 1999). In *P. desertorum*, workers are on average 2.8 times more closely related to females than to males. Yet, investment ratio is very nearly equal (1.01:1 female:male), consistent with substantial queen control. Workers recognize the sex of the brood and, as predicted from within-colony relatedness asymmetry, they discriminate against males in favor of females when they are given immature individuals of both sexes (HELMS & al. 2000). Male elimination is independent of whether workers come from female- or male-specialist colonies. This strongly suggests that sex ratio specialization in this species results from the fact that queens in about half the colonies constrain workers to rear only males among sexual offspring. As mentioned above, this may be achieved in two complementary ways: by biasing the primary sex ratio during the period when reproductive female production can be initiated, and / or by controlling female caste determination by primarily laying worker-destined eggs rather than queen-destined eggs.

Such a dual system of queen influence over colony sex ratio has been demonstrated in the ant *Pheidole pallidula*. Colonies are headed by a single or several unrelated queens (FOURNIER & al. 2002); as a consequence, RA are uniformly maximal in all colonies and workers should favor a female-biased sex ratio. Even so, colonies produce a single gender of reproductives and the population-wide sex-investment ratio lies between the queen and worker optima (1.1:1 to 1.8:1 female:male; KELLER & al. 1996a, ARON & al. 1999a, FOURNIER & al. 2003). Sex specialization is tightly associated with breeding structure, with monogynous colonies producing a male-biased brood and polygynous colonies almost exclusively a female-biased brood. The probable causes for this reproductive pattern are explained below (see Competition among queens over sexual production). By combining genetic and endocrinology studies, de MENTEN & al. (2005b) showed that queens exert control over colony sex ratio in two ways. First, queens from monogynous (male-producing) colonies lay a primary sex ratio significantly more male-biased than that of queens from polygynous (female-producing) colonies, at the period of sexual production. This ratio is sustained as eggs mature into larvae. Second, queens influence the caste fate of the diploid eggs they produce by hormonally biasing their development into either a worker or reproductive form. Caste determination in *P. pallidula* occurs during oogenesis and depends to some extent on the level of juvenile hormone (JH-III) produced by the queen; high level of JH-III promotes sexualization of the female brood (PASSERA & SUZZONI 1979, PASSERA 1980). Caste ratio analyses during brood development show that most diploid brood develops into sterile workers in monogynous colonies, whereas a large proportion of diploid brood develops into reproductive females in polygynous colonies. Accordingly, queens from polygynous, female-producing nests produce JH-III at a significantly higher rate than queens from monogynous, male-producing nests. In short, although workers of *P. pallidula* have the ability to eliminate males and bias sex investment ratio (KELLER & al. 1996a), the magnitude of their control is largely constrained by the proportion of male eggs together with the proportion of queen-destined eggs produced.

Data from other ants also showed the influence of maternal effects on the developmental fate of female brood. New queens of *Pogonomyrmex* harvester ants develop only from fertilized eggs laid by mothers that are at least two years old and that were previously exposed to cold (SCHWANDER & al. 2008). Moreover, eggs developing into queens are characterized by a significantly lower level of ecdysteroids than eggs developing into workers. By influencing caste determination of their female offspring, queens may potentially control reproductive decisions of the colony.

(III) Primary sex ratio bias and control of caste ratio in parthenogenetic ants: Another remarkable reproductive mechanism that allows queens to control caste ratio has been reported for several ant species, *Cataglyphis cursor* (PEARCY & al. 2004), *Wasmania auropunctata* (FOURNIER & al. 2005), *Paratrechina longicornis* (PEARCY & al. 2011), *Vollenhovia emeryi* (OHKAWARA & al. 2006) and in genetically recombinant populations of *Mycocepurus smithii* (RABELING & al. 2011). In these species, queens use alternative modes of reproduction for the worker and queen castes. Workers are produced by normal sexual reproduction, whereas new queens are produced by thelytokous parthenogenesis from unfertilized eggs. In three out of the five species above, caste determination is influenced by genetic effects (SCHWANDER & al. 2010). In *W. auropunctata* and *P. longicornis*, genetic caste determination is quasi-perfect, since virtually all workers arise from sexual reproduction and all queens from parthenogenesis (FOUCAUD & al. 2010, PEARCY & al. 2011). Moreover, both castes are produced at the same timing in the year, excluding seasonal production of sexual and parthenogenetic eggs. A similar situation occurs in *V. emeryi*; however, in this species the phenotype-genotype match is slightly weaker because up to 5% of the workers develop from parthenogenetic rather than from fertilized eggs.

Two studies have documented a possible control by queens over the colony secondary sex ratio through caste determination of female eggs in parthenogenetic ants. In *Cataglyphis cursor*, female caste is not inherently determined at diploidization (parthenogenesis or fertilization). It relies on the nutritional state of the larvae, and diploid larvae can be diverted from their developmental fate until the third larval instar (CAGNIANT 1982), so that new queens can arise from fertilized eggs. Nonetheless, mothers maximize their fitness by actively regulating the proportion of fertilized and parthenogenetic eggs laid over time. Thelytokous eggs are produced in early spring, when workers raise the diploid brood into sexuals. The proportion of parthenogenetic eggs laid decreases rapidly, and after about three weeks all diploid eggs arise from sexual reproduction and give rise to non-reproductive workers (ARON & al. 2011). This temporal adjustment of caste-ratio by queens directly affects the primary sex ratio, and hence the secondary sex ratio, in *C. cursor*.

In the short-winged form of the Japanese ant *Vollenhovia emeryi*, both male and female sexuals are produced clonally (OHKAWARA & al. 2006). New queens develop by thelytokous parthenogenesis and inherit their whole genome from their mother, whereas males are produced from fertilized eggs after exclusion of the maternal nuclear DNA (they thus bear only the paternal genome). Workers arise from diploid, fertilized eggs. This reproductive system gives rise to specific patterns of relatedness within colonies: A queen is related to her female sexual, worker, and male offspring by 1.0, 0.5, and 0, respectively. In terms of relatedness, queens may therefore optimize their fitness by producing mostly female sexuals (and workers for colony efficiency). Males are required only to ensure mating partners for female offspring in order to produce workers. Consistent with relatedness values, queens invest more resources in producing queen-destined eggs than male- or worker-destined eggs (OKAMOTO & OHKAWARA 2010). The more sexual eggs are laid, the more queen-destined eggs are produced. Interestingly, the proportion of female eggs is negatively correlated to that of worker eggs, indicating a trade-off in the production of both female castes.

Competition among queens over sexual production: Analysis of primary sex ratio variations over time indicates that, in several ant species, a significant proportion of haploid eggs are produced all year round. Even outside sexual production times, when no males are reared, the percentage of male eggs laid by queens rarely falls below 10% (e.g., *Camponotus nipponicus*, HASEGAWA 1992; *Linepithema humile*, ARON & al. 1994; *Solenopsis invicta*, ARON & al. 1995; *Pheidole pallidula*, KELLER & al. 1996a; *Formica exsecta*, SUNDSTRÖM & al. 1996; *Plagiolepis pygmaea*, ARON & al. 2004). The continual production of haploid eggs may reflect constraints on efficiency of egg fertilization, or it may serve functions such as food for larvae and workers (NONACS 1991).

However, in some species, haploid eggs are produced in such great numbers that other explanations were proposed. KELLER & al. (1996b) hypothesized that the strategy would allow queens to achieve higher reproductive success when colonies lose most or all their queens and must rear new sexuals from the brood present within the colony. This hypothesis was tested in the Argentine ant *Linepithema humile*, where primary sex ratio is almost always above 0.30 (ARON & al. 1994). Colonies of *L. humile* are headed by a large number of unrelated queens. Outside the period of sexual production, workers cannibalize all the haploid brood, whereas the diploid brood develops into sterile workers (PASSERA & ARON 1996). However, orphaning of the colonies triggers sexualization of the diploid brood, and production of both male and female sexuals at any time of the year (VARGO & PASSERA 1992). Males and females readily mate in the nest, thereby providing a rapid mechanism of queen replacement (PASSERA & al. 1988, KELLER & PASSERA 1992). New colonies of *L. humile* arise through budding or from passive transport of colony fragments (either natural or by human trade), and batches of workers and brood only are probably frequent (PASSERA 1994). Moreover, queens and workers frequently move between nests, resulting in important variation in queen number per colony. KELLER & al. (1996b) predicted that selection should favor continual production of male eggs in the event the colony (or fragment) becomes queenless. Ultimately, the proportion of haploid eggs laid by each queen should depend on the number of queens in each nest. The higher the number of queens producing males, the higher the probability of competition between sons from several queens for access to females and, hence, the higher the selection on each individual queen to produce a larger fraction of males for increasing her fitness. Conversely, queens should lay fewer haploid eggs when there is a decreasing probability that their sons will compete with those of other queens. Note that because nestmate queens are on average unrelated, the effect of LMC over sex ratio is relaxed. By comparing the proportion of haploid eggs laid by queens from single-queen and multiple-queen experimental colonies, KELLER & al. (1996b) showed that queens of *L. humile* adjust the primary sex ratio according to queen number, as predicted: Females in multiple-queen colonies lay significantly more haploid eggs than females in single-queen colonies. Thus, mothers lay a higher proportion of haploid eggs when their sons are more likely to compete with those of other queens.

In *Pheidole pallidula*, competition among nestmates queens results in overproduction of female sexuals. As mentioned previously, colonies of *P. pallidula* are headed by a single or several unrelated queens; sex ratio distribution is bimodal, with monogynous colonies producing a male-biased brood and polygynous colonies a female-biased brood. That female sexuals are mainly produced by polygynous colonies is unusual in ants, and might stem from the rivalry among queens (FOURNIER & al. 2003). In colonies with multiple unrelated queens, they compete to be mother of as much of the offspring as possible. The ensuing overabundance of eggs may allow workers to manipulate sex ratio to their own advantage. Because queens are unrelated, relatedness asymmetry within colonies is high and workers should favor a female-biased sex ratio investment. As a result, queens in monogynous colonies would benefit to produce mainly or only males. Primary sex ratio studies showed that queens can force workers to raise males by limiting the number of diploid eggs laid and by hormonally controlling their caste fate (DE MENTEN & al. 2005b). Overall, this leads to split sex ratio in the population.

Future directions

The number of studies on sex allocation in social Hymenoptera has increased dramatically over recent years and many factors have been reported to correlate with skews in male and female investment. Whether queens modify or can adjust primary sex ratio and / or the caste ratio in relation to genetic and / or environmental factors remains, however, largely unexplored. Few works were performed on ants and bees; to the best of my knowledge, nothing has been documented for wasps. A crucial barrier to progress in the field has been the difficulty in determining the proportion of haploid eggs laid by queens. Recent developments in molecular techniques, allowing fast and easy sex determination even at the egg stage, open up a potentially fruitful research area on primary sex ratio regulation and, possibly, on how queens influence – or attempt to influence – caste ratio among diploid offspring.

Possible issues for primary sex ratio studies:

Do queens adjust the primary sex ratio in response to the strength of local competition? In contrast to the numerous and rigorous studies of sex ratio in haplodiploid parasitoids and fig-pollinating wasps, few studies have explicitly tested facultative adjustment of the sex ratio by queens in response to local competition in ants and other social Hymenoptera. Under LMC, theory predicts that the queens and the workers should favor a female-biased sex ratio. However, a conflict still exists between them because the stable secondary ratio diverges for each female caste, with workers always preferring a more female-biased ratio than queens (BOURKE & FRANKS 1995). Whether queens control the proportion of male and female eggs laid in response to the strength of local mate or local resource competition certainly deserves in-depth investigations. Moreover, comparison between the primary and secondary sex ratios under various intensities of local competition would shed light on the respective contribution of queens and workers on colony sex ratio adjustment.

Do queens adjust the primary sex ratio depending on environmental conditions? Experimental manipulation of social and environmental conditions, such as colony demography or resource availability, are relatively easy to carry out in the laboratory and should also help in determining the extent of queen ability to adjust the primary sex ratio. Resource availability may be of particular interest, because it has been shown to be a primary factor of sex allocation patterns in social Hymenoptera. Colony-level food abundance may be determined by both extrinsic factors (e.g., temporal environmental stochasticity, variation in availability or quality of food across patches) and intrinsic factors (e.g., colony age and size). Several studies have shown a positive association between reproductive output and relative investment in females in various ants, including *Formica podzolica* (DESLIPPE & SAVOLAINEN 1995), *Aphaenogaster rudis* (MORALES & HEITHAUS 1998), *Pheidole pallidula* (ARON & al. 1999a), *Messor pergandei* (ODE & RISSING 2002), *Myrmica brevispinosa* (BONO & HERBERS 2003), *Formica exsecta* (BROWN & KELLER 2006), *Aphaenogaster smythiesii japonica* (IWANISHI & al. 2007), as well as in the honeybee *Apis mellifera* (FREE & WILLIAMS 1975, VELTHUIS & al. 1990). Such a positive relationship between total sexual productivity and female production is predicted by the resource abundance hypothesis (NONACS 1986a,b, ROSENHEIM & al. 1996), which assumes that food-constrained colonies should opt for colony growth (worker production) as an investment in survival until better times and a male bias follows as a by-product, whereas well-fed colonies can convert a large proportion of the diploid brood into reproductive females and should rear both female sexuals and males with a relative overinvestment in winged females. The effect of the amount of food resources on colony sex ratios could contribute to natural selection favoring a shift in the primary sex ratio laid by mother queens. Queens under poor conditions might be expected to shift sex ratio and / or caste ratio at the egg stage with food availability. SASAKI & OBARA (2001) provided the first evidence that honeybee queens adjust the egg sex ratio depending on the nutritional status of their colony. In the reproductive season, queens from well-fed colonies lay more than twice the amount of eggs than queens from poorly-fed colonies. The proportion of male eggs laid is six times higher when food is abundant than under food shortage (0.07% vs. 0.44%). Outside the reproductive period, the total number of eggs laid by queens is not different between the two feeding conditions and no difference in the primary sex ratio laid by queens occurs (0.04 *vs*. 0.01). Such experiments have not been performed in ants so far. In the same vein, whether ant queens manipulate the caste fate of female eggs at the period of sexual production (by changing egg hormone level, or by controlling the proportion of diploid eggs produced through sexual and thelytokous reproduction) in response to the availability of food resources remains unstudied.

Other stressful environmental conditions could also potentially affect primary sex ratio. For example, social insects exploit environments that make them susceptible to infection and rapid disease transmission. Parasites and pathogens may have devastating effects on colony performances and reproductive success (SHYKOFF & SCHMID-HEMPEL 1991, SCHMID-HEMPEL 1998, BAER & SCHMID-HEMPEL 1999). It has been suggested that parasite pressure could select for increased genetic diversity among progeny, outbreeding and dispersal from infected areas (SHERMAN & SHELLMAN-REEVE 1994, SHERMAN & al. 1998). In particular, a male biased brood would promote both extreme dispersal and outbreeding, because males are usually much smaller and lighter than reproductive females. According to this hypothesis, increased investment in males would benefit both the queen and the workers, and queens should adjust the primary sex ratio in response to the local severity of infection (SHERMAN & SHELLMAN-REEVE 1994). Thus far, no evidence for an effect of infestation by parasites and pathogens on colony sex ratio has been documented in ants and other social insects.

Informative cues for primary sex ratio adjustment: The ability of queens to adaptively bias the primary sex ratio depends upon the quality of information queens have about their environment. Queens may potentially use extrinsic cues (temperature, variation in resource availability or quality, …) and / or intrinsic cues (e.g., nest architecture, number of reproducing queens in the colony, presence of eggs laid by other females, average relatedness of queens with workers in the nest, colony size, …) to process relevant information about their environment. How these factors are assessed and responded to is largely unknown, and their elucidation remains an important task.

Both field and laboratory studies strongly suggest that ant queens use temperature variations as a proxy to assess seasonal changes (HASEGAWA 1992, ARON & al. 1994, ARON & al. 2011). In the honeybee, cell type provides queens an indication of the type of eggs (fertilized or unfertilized) that they should lay. Queens accurately assess the type of cell (worker or drone cell) in which they are laying, and precisely control the fertilization of their eggs (KOENIGER 1970, SASAKI & al. 1996, RATNIEKS & KELLER 1998): All eggs laid in drone cells are haploid, whereas all eggs laid in worker cells are diploid. Although workers influence investment in drones because they build the wax cells, queens also contribute to colony reproductive decisions by adjusting the proportion of drone eggs based on the amount of haploid eggs laid previously (WHARTON & al. 2007). Remarkably, the ability to choose the ploidy of their eggs was also reported for virgin queens of the Cape honeybee *Apis mellifera capensis* (OLDROYD & al. 2008). Here, unmated queens produce diploid-female and haploid-male eggs, respectively, by thelytokous and arrhenotokous parthenogenesis. Genetic analyses show that ca. 95% of male or female eggs are laid in the correct cells. This indicates that virgin Cape honeybees have at least partial control over the kind of meiosis their eggs undergo. According to OLDROYD & al. (2008), queens could influence the second-division meiosis that occurs in each egg after it has been laid, by encoding a signal in the egg at the laying time. Ant queens do not lay their eggs in cells; nest structure does not provide a cue for primary sex ratio determination.

How queens of social insects assess colony structure and the strength of local competition also remains uncertain. In fig-pollinating wasps, females adjust the sex ratio of their offspring in response to the number of females that lay eggs in a patch (HERRE 1985, 1987). In the parasitoid wasp *Nasonia vitripennis*, the presence of eggs laid by other females provides a reliable cue allowing females to assess the intensity of local mate competition and adaptively adjust offspring sex ratio (SHUKER & WEST 2004). Likewise, queens of social Hymenoptera could use the actual number of reproducing females in their colony or the presence of brood from other queens as an informative cue to estimate colony structure. They could also perceive the presence of other reproductive queens based on chemical cue diversity in their colony. Hydrocarbon profiles of the wax layer on the cuticule have been shown to be correlated with a wide range of social behaviors in insects, such as kin and nestmate recognition, queen fertility signaling or task performance (LENOIR & al. 2001, HOWARD & BLOMQUIST 2005). Variation in hydrocarbons is also an accurate predictor of sex ratio variation in single-queen colonies of the wood ant *Formica truncorum*, a species where queens have multiple matings (BOOMSMA & al. 2003). In colonies where different patrilines are characterized by distinct hydrocarbon profiles, workers correctly assess the number of times that their queen has mated and rear a male biased brood (as predicted from split sex ratio theory). By contrast, in colonies where the different patrilines show similar hydrocarbon profiles, workers tend to incorrectly assess the mating frequency of their queen, and rear a female-biased or unbiased brood. Whether queens use variations in hydrocarbons or other chemical cues to detect the presence of other laying queens in their colony has not yet been investigated.

Constraints on adaptation for primary sex ratio adjustment: Both field and laboratory experiments reveal variations around predicted optimum sex ratios in solitary and social Hymenoptera. Such variations can reflect differences in environmental conditions, in social conditions, in availability of informative cues, or they may stem from a combination of several of these factors. The occurrence of multiple – sometimes opposing – factors jointly influencing sex ratio decisions in social species might preclude the existence of a single sex ratio optimum (KÜMMERLI & KELLER 2011).

Sex ratio variations may also be genetically determined. Although our understanding of how natural selection should shape sex allocation is straightforward, the genetic bases of sex ratio determination remain largely unknown (WEST & HERRE 2002, WEST & SHELDON 2002). Given the close link between offspring sex ratio and individual fitness, the genetic bases of sex ratio behavior and, ultimately, the genes affecting sex ratio adjustment certainly merit further examination. PANNEBAKKER & al. (2008) provided the first study on the genetic basis of sex ratio, and how genetic architecture may potentially constrain adaptive sex ratio, in the parasitoid wasp *Nasonia vitripennis*. By using a mutation accumulation experimental approach, they showed that mutations generate variation in sex ratio at the adult stage. However, genetic variance of sex ratio is lower than expected under a mutation-selection balance, suggesting additional selection against sex ratio mutations. The authors then identified in *N. vitripennis* the first quantitative trait locus (QTL) for sex ratio variation reported in any organism (PANNEBAKKER & al. 2011). Another issue related to genetic constraints on sex ratio concerns the influence of genomic imprinting, i.e., differential expression of maternally and paternally derived alleles (BURT & TRIVERS 2006). Social Hymenoptera represent a fertile ground for genetic conflicts between parents for at least two reasons: (1) The haplodiploid determining system means that fathers make a genetic contribution only to daughters, and (2) worker offspring can manipulate sex allocation through male killing or biasing the final caste of developing females. This should greatly promote selection for genomic imprinting, with paternally inherited alleles inducing workers to favor a more female-biased sex allocation (HAIG 2000, QUELLER 2003, WILD & WEST 2009).

To date, there is a critical shortage of data on the genetic bases of sex allocation, including whether imprinting occurs in genes involved in sex ratio determination, in ants and other social Hymenoptera. Some studies are, however, consistent with a heritable component (genetic or maternal effects) in sex ratio and caste determination in ants. KÜMMERLI & KELLER (2007) first reported a pattern of reproductive specialization among nestmate queens in the ant *Formica exsecta*, where 84% of all queens contribute exclusively to one type of brood (either males or workers). Differences in lifetime sex ratios among queens were also documented in *Cardiocondyla kagutsuchi* (FROHSCHAMMER & HEINZE 2009). In this species, queens from lineage A produce a significantly more female-biased secondary ratio and female larvae with a significantly higher queen bias (female sexuals / all females), than queens from lineage B. Co-existence of these two genetic lineages can be evolutionarily stable if queens belonging to each lineage have the same average fitness. Recently, strong effects of the maternal and paternal lineages on offspring production and caste allocation were found in the Argentine ant *Linepithema humile* (LIBBRECHT & al. 2011). There is a significant effect of the paternal lineage on the proportion of females developing into queens or workers, and a significant interaction between parental lineages for the proportion of offspring being queens or males.

Conclusions

Social Hymenoptera have provided some of the most convincing support for the broad predictions of sex allocation theory. A number of studies have provided cases in which there is quantitative fit to theory such as LMC, LRC and, more generally, to kin selection and social conflict theory. However, colony sex ratio depends on two parties – the queen(s) and the workers – and their relative contribution to colony sex ratio adjustment remains poorly studied. Workers have been shown to manipulate sex allocation of reproductives in a number of species. In contrast, less is known about the ability of queens to influence reproductive decisions. Queens have, however, several mechanisms available to regulate colony sex ratio: Haplodiploidy allows queens to control the proportion of male and female offspring produced; and queens of some species influence the caste fate of female eggs by modulating egg hormone level (JH, ecdysteroids) or by using selectively sexual reproduction and thelytokous parthenogenesis for the production of the non-reproductive and reproductive castes. Clearly, there is much potential for future work in this area, examining whether and how queens adjust the proportion of male and female eggs laid in response to ecological, demographic and social factors both within and across species, through a combination of theoretical, behavioral, physiological and genetic approaches.

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