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Conflict and co-operation in ant societies

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*Anche le formiche,
nel loro piccolo,
s'incazzano.*

Gino e Michele

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Chapter 1

Introduction

Associations between biological entities are ubiquitous in nature (Herre *et al.*, 1999). Some are maintained over long evolutionary time-spans, such as the alliances of genes on chromosomes, eucaryotic genomes and mitochondria, cells in multicellular organisms, or of the members of animal societies. Other associations are of more transient nature. These include reproducing pairs of animals, horizontally transmitted parasites and their hosts, or predators and their prey.

The coalescence of formerly independent units into associations creates the opportunity for co-operation but also conflict. Co-operation occurs whenever a member of a group acts so as to increase the productivity and survival of the group as a whole. Co-operation can be associated with division of labour whereby group members specialise in the tasks they perform (Maynard Smith and Szathmary, 1995; Queller, 2000). Different tissues of a metazoan body, for example, fulfill different functions. Analogously, the different tasks in a social insect colony are often performed by individuals of different castes which can have differentiated morphologically. However, in most cases biological alliances also involve conflict, because the interests of all group members do not completely coincide. Selfish strategies can occur that increase individual fitness at the expense of the fitness of other group members (Leigh, 1977; Queller, 2000; Herre *et al.*, 1999). The example of parasites and their hosts demonstrates this very well: strategies that increase parasite reproduction often have deleterious effects on host fitness. Inversely, a more efficient immuno-response will benefit the host's fitness but diminish that of the parasite whose survival and/or reproduction are decreased.

Co-operative and antagonistic strategies are selected at different hierarchical levels. Selection at the level of the association as a whole favours increased overall productivity and therefore bolsters co-operation. Selection within groups, on the other hand, is concerned solely with the fitness of indi-

vidual group members and favours strategies that increase individual success, even if it is achieved against the interests of other group members. Whether it is co-operation or conflict that dominates the interactions between the members of an association thus depends on the relative strength of higher- and lower level selection. Two situations where selection at the higher level is very important are groups of closely related individuals and associations between vertically transmitted symbionts and their hosts. In both cases, individual and group success are coupled and high degrees of co-operation can evolve. If group members are genetically very similar, there is not much incentive for individuals to promote their own genes at the expense of group-mates, because these will share a great proportion of alleles. Selection will therefore tend to favour co-operative strategies, unless the increase in productivity due to co-operation results in increased competition among the offspring of the group (Queller, 1992, 2000). Two examples of highly co-operative kin-groups are the metazoan body and social insect colonies. In both, we observe division of labour that involves even the reproductive tasks, accomplished by specialised members of the group, the germ line of our body and queens and males in social insects. In vertically transmitted symbionts, the coupling of fitness between group members results from the fact that symbionts can only attain the offspring generation in association with host progeny. They are therefore selected for a benign relationship with their hosts in order to assure an optimal reproduction of the latter. An example of such a positive association are Buchnera, symbiont bacteria found in aphids. In this case, both partners rely on the alliance, Buchnera benefit from the shelter and resources supplied by their host, whereas the aphids depend on amino acids synthesised by their symbionts (Douglas, 1996).

While genetic relatedness or coupled reproduction can favour the evolution of elaborate co-operation, conflict often persists. This thesis investigates conflict in two animal associations, Hymenopteran societies and the association of *Wolbachia* symbionts and their hosts. Social Hymenoptera are one of the prime examples for co-operation in the animal kingdom. In their colonies, reproduction is usually monopolised by one or several queens, while workers forgo reproduction and perform tasks like nest-construction, foraging, and brood care. Despite the apparent harmonious co-operation, Hymenopteran societies are also the scene of conflicts. The best studied of these is queen-worker conflict over sex allocation. As first pointed out by Trivers and Hare (1976), conflict results from relatedness asymmetries caused by the haplodiploid mechanism of sex determination. In haplodiploid species, females develop from fer-

tilised diploid eggs, whereas males develop from unfertilised haploid eggs. As a consequence, in a colony headed by one singly-mated queen, workers are three times more related to female sexuals (their sisters, $r = 0.75$) than to male sexuals (their brothers, $r = 0.25$). Reflecting this asymmetry in relatedness, equilibrium sex allocation under worker control is female-biased, with three times more resources being invested in new queens than in males. Queens, in contrast, are equally related to sons and daughters and therefore favour an even investment in the two sexes.

Since Trivers and Hare, the body of theory on queen-worker conflict over sex allocation has grown considerably (Bourke and Franks, 1995; Crozier and Pamilo, 1996). Notably, predictions of equilibrium sex allocation under queen and worker control of investment have been generated for a wide range of social and ecological contexts. However, apart from some exceptions, these studies have concentrated on the within-colony level, investigating the optimal selfish strategies of workers and queens. The effects of queen and worker manipulation on the productivity of the colony as a whole were not taken into account. The first part of this thesis attempts to fill this gap. Chapter 2 presents a very general model of optimal sex allocation under worker control that includes costs of sex ratio manipulation. In order to control sex allocation, workers have to adjust the egg sex ratio, controlled by the queen who chooses whether to fertilise or not the eggs she lays. The manipulation of brood sex ratio by workers is likely to incur some loss of resources and thereby affect colony productivity. The evolution of sex allocation under worker control will therefore be the result of selection on the within-colony level (mediated by relatedness asymmetries) as well as on the between-colony level (mediated by costs of sex ratio manipulation). Chapter 3 considers a specific mechanism of sex ratio biasing, the manipulation of female cast. Because workers as well as female sexuals develop from diploid eggs, workers can increase the investment in females by raising more female brood as sexuals. The increased female bias in sex allocation is associated with a reduction of the colony's worker force and thus imposes costs in terms of colony productivity. Chapter 3 investigates how the balance of the two opposed effects determines sex ratio evolution. Whereas Chapter 2 concentrates only on worker behaviour, Chapter 3 includes both players in the conflict, queens and workers. The model explicitly considers selection acting on the two parties to gain control over investment and explores the possible equilibrium where control is shared between queens and workers. Finally, I complete the analysis by considering the evolutionary interests of the developing female larvae themselves which

have the choice between the altruistic strategy of worker development and the selfish one of becoming queens. Again, the evolution of larval strategies are analysed in interaction with possible counter-manipulation by queens or workers.

The second part of my thesis concentrates on the association between the symbiont *Wolbachia* and its ant host *Formica exsecta*. *Wolbachia* are alpha-proteobacteria that live in the cytoplasm of many arthropod species (Werren, 1997). In exactly the same way as mitochondria, they are transmitted vertically from the mother to the offspring. On the basis of its vertical mode of transmission, one would expect *Wolbachia* to have benign or positive effects on its host. This is true in the sense that *Wolbachia* seldom have significant effects on the fertility of infected hosts. However, in the same way as hymenopteran queens and workers, *Wolbachia* and their hosts disagree over the relative value of male and female offspring of the host (Werren and O'Neill, 1997). Whereas the host transmits genes equally through both sexes, males represent a dead end for the maternally transmitted symbiont. Regularly, *Wolbachia* appear to override the interests of their hosts. In many host species, the symbiont induces drastic alterations of host reproductive physiology, all of which promote the spread of *Wolbachia* infection at the expense of the transmission of host genes through males. In many cases the production of male offspring is completely suppressed. In isopods, for example, *Wolbachia* has a feminising effect, causing genetic males to develop into functional females (Rousset *et al.*, 1992b; Rigaud *et al.*, 1991). In several species of parasitic wasps, *Wolbachia* induces females to reproduce parthenogenetically, which equally results in all-female broods (e.g. Stouthamer *et al.*, 1993). In other species, such as ladybirds and butterflies, *Wolbachia* causes the selective abortion of male offspring during early development (Hurst *et al.*, 1999). In hosts in which offspring compete for resources, this will allow for increased survival of females. Alternatively to influencing host sex allocation directly, *Wolbachia* can take advantage of males as a means to impede reproduction of un-infected females (Caspari and Watson, 1959; Turelli and Hoffmann, 1991). Sperm of infected males contains factors that prevent egg development unless the egg contains *Wolbachia*. This "cytoplasmic incompatibility" between infected males and un-infected females gives an advantage to infected females, who can reproduce normally with any male.

Given *Wolbachia*'s interest in biasing host reproduction towards females, Chapter 4 attempts to relate variations in sex allocation between colonies to variations in infection with *Wolbachia*. In populations of the ant *Formica ex-*

secta, the sex ratio of sexuals produced by colonies is bimodally distributed. Most colonies produce almost exclusively male sexuals, whereas a minority of colonies produces sexuals of both sexes. The hypothesis tested in Chapter 4 is that female production is induced by *Wolbachia* symbionts. The prevalence of infection is therefore expected to be higher in colonies that produce females than in those specialising on males. This study is important in the wider context of this thesis. First, it investigates possible points of conflict between host and symbiont. If female-production was indeed found to be due to manipulation of the host by *Wolbachia*, this would indicate that host and symbiont are in disagreement over reproduction. Second, it could have implications for the interpretation of sex ratios. Demonstrating the influence of endosymbionts on sex allocation would suggest that the reasons for variations in sex allocation have not always to be sought in the genetic structure of the ant colonies.

Finally, Chapter 5 investigates the association between *Wolbachia* and host mitochondria. A joint analysis of mitochondrial haplotype of hosts and the *Wolbachia* strains they carry allows to verify the faithfulness of vertical symbiont transmission. Pure vertical transmission can be detected by a perfect genetic linkage of host mitochondria and *Wolbachia*, which is disrupted by horizontal symbiont transfer between mitochondrial host lineages (Hoffmann and Turelli, 1997). Furthermore, investigating the linkage between host mitochondrial haplotypes and the *Wolbachia* strains they carry is of special interest in *F. exsecta*. The reason is that rates of female dispersal are extremely low in this ant (Liautard and Keller, 2001). Consequently, mitochondrial genotypes are strongly differentiated between ant populations. If *Wolbachia* was effectively purely maternally transmitted this creates the interesting possibility of symbionts showing a population genetic structure identical to that of the host.

Chapter 2

Levels of selection in hymenopteran sex ratio conflict

The study described in this chapter has been conducted in collaboration with Ken R. Helms and Laurent Keller. The manuscript is in preparation for submission to *The American Naturalist*.

Introduction

Queen-worker conflict over sex allocation has been an important area of research on social insects over the past 25 years. As first outlined by Trivers and Hare (1976), queens and workers in a hymenopteran colony differ in their optimal sex allocation. This difference is caused by relatedness asymmetries arising from haplodiploidy, the hymenopteran system of sex allocation. In haplodiploid species, females develop from fertilised (diploid) eggs whereas males develop from unfertilised (haploid) eggs. As a consequence, in a colony headed by one singly-mated queen, workers are three times more related to female sexuals (their sisters, $r=0.75$) than to male sexuals (their brothers, $r=0.25$). Corresponding to this asymmetry in relatedness, equilibrium sex allocation under worker control is female-biased, with three times more resources invested in females than in males. Queens, on the other hand, are equally related to sons and daughters and therefore favour an even investment in the two sexes.

Since Trivers and Hare, hymenopteran sex ratio theory has been greatly developed. Predictions of optimal sex allocation under queen and worker control have been generated so as to include more complex genetic structure of colonies and populations (Bourke and Franks, 1995; Crozier and Pamilo, 1996). While incorporating great genetic and ecologic complexity, all these models were based on the unrealistic assumption that workers can attain their equilibrium sex ratio without any cost. This assumption is likely to be wrong in most cases. Adjusting sex allocation requires that workers manipulate the queen-controlled egg sex ratio. Two mechanisms have been described how they can do so. First, workers can eliminate male brood and invest the released resources in the production of more females. This mechanism has been found to be used by workers of the ant *Formica exsecta* (Sundström *et al.*, 1996). But in this species the elimination of males has also been shown result in a loss of resources, because energy already invested in the individuals killed cannot be completely recovered (Chapuisat *et al.*, 1997). Additional costs may arise if the timespan between elimination of males and the end of the reproductive season is too short for all replacement females to complete development. A second mechanism allowing workers to adjust sex allocation consists of influencing the caste determination of female brood. In many species, the quantity and/or quality of nutrition during development determines whether females become workers or gynes. Workers can use the mechanism of caste determination for adjusting sex allocation by manipulating the number of females reared as sex-

uals. Workers of the ant *Leptothorax acervorum* have been shown to control colony sex allocation in this way (Hammond *et al.*, 2002). Sex ratio manipulation via female caste determination is almost certainly associated with costs. Raising more females as gynes reduces the investment in new workers, thereby compromising colony growth and productivity.

In this paper we incorporate the costs imposed by the workers' brood manipulation in a general model of sex ratio evolution under worker control. In a first model we assume that cost is identical in all colonies. We show that sex ratio manipulation is under the influence of two opposing selective forces. Individual-level selection, mediated by the difference in relatedness to male and female sexuals, acts in favour of sex ratio biasing. On the other hand, colony-level selection acts against sex ratio biasing, because the costs of brood manipulation reduce colony productivity. The resulting equilibrium sex allocation can fall short of the 3:1 female-bias predicted by Trivers and Hare. In a second model we explore how variations in cost between colonies influence colony sex allocation. Variations are expected for example due to spatial fluctuations in resource availability which influence the cost of replacing eliminated male brood. We predict that colonies differing in cost will diverge with respect to the sex ratios they produce, whereby colonies in which sex ratio manipulation is less costly will produce disproportionately female-biased sex ratios whereas colonies in which manipulation is more costly will reduce female bias in sex allocation.

The Model

Our model investigates the evolution of sex allocation among the sexuals produced by colonies of social Hymenoptera. We consider a large population of colonies. Each colony is composed of one queen and workers. The queen produces a primary sex ratio p , defined as the proportion of females among sexual brood. Workers alter the egg sex ratio, changing the proportion of females by m_i . We assume that workers do so collectively and will not consider variations in behaviour among workers of a same colony. The sex ratio of sexuals produced by colony i is therefore given by $s_i = p + m_i$. As our analysis focuses on the evolution of worker behaviour, we assume the primary sex ratio p to be invariable. In numerical calculations, we will fix its value to the queen's equilibrium sex ratio $p = 0.5$. Fixing p to different values below $p = 0.75$ would not change our results qualitatively.

Sex ratio manipulation is assumed to entail a cost in terms of colony productivity which is reduced by a proportion $\gamma(m_i) = m_i^a$. Colony productivity is thus given by $P_i = 1 - \gamma(m_i)$. The parameter a determines the form of the cost function. We assume values of $a \geq 1$, corresponding to an increase of cost with manipulation which is either linear ($a = 1$) or exponential ($a > 1$). In the latter case, greater values of a result in a cost function that increases more slowly over the range of m considered here (Fig. 2.1). The assumption of exponential cost can be justified for both mechanisms of brood manipulation. The manipulation of female cast will cause an exponential cost, because of its negative impact on worker production. In several species, colony productivity has been shown to be a saturating function of worker number (e.g. Brian, 1956; Tschinkel, 1993; Sundström, 1995). In this case, reducing the colony's worker force by raising female brood as sexuals decreases productivity at an accelerating rate. Also the elimination of males as a means of sex ratio adjustment can cause exponential costs. This should be the case, for example, when morphological differences among males generate variance in how easily males can be identified as such. Small alterations of sex ratio can then be achieved by eliminating males whose sex can be easily determined. Accomplishing a stronger bias in sex allocation, however, will incur much higher costs because it involves the culling of brood whose sex is more uncertain. This implies an elevated risk of eliminating females by accident.

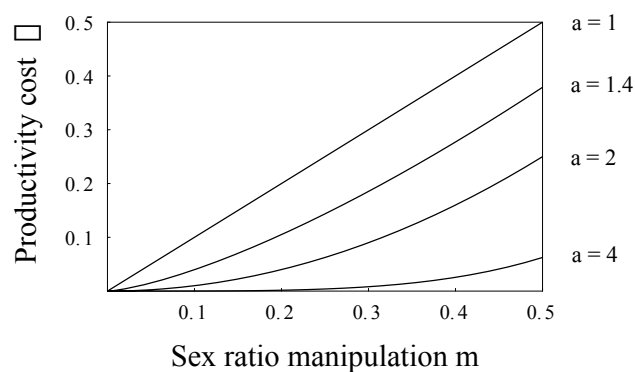


Figure 2.1: Reduction of colony productivity γ as a function of the degree of sex ratio manipulation m . Cost functions are shown for different values of a .

We assume that the sexuals produced in the population mate randomly with the mating success of a given sex being proportional to the inverse of the

population mean investment in that sex (e.g. Crozier and Pamilo, 1996). The fitness of a member of colony i gained through males (W_m) and females (W_f) is proportional to the product of colony productivity, the colony's investment in a sex, and mating success, $W_m = P_i(1 - s_i)/(1 - s)$ and $W_f = P_i s_i/s$, where $s = p + m$ is the mean investment in females in the population.

Based on the above assumptions we can express the fitness of workers in colony i as the sum of fitness gained through males and females, weighted by the two sexes' reproductive values c_m and c_f ,

$$W_i = c_m W_m + c_f W_f . \quad (2.1)$$

The evolutionary change in sex ratio manipulation is given by the derivative of worker fitness (Eq. 2.1) with respect to a random genotype g' in the descendant generation (Taylor and Frank, 1996; Frank, 1998)

$$\begin{aligned} \frac{dW_i}{dg'_i} &= c_m \frac{dW_m}{dg'_m} + c_f \frac{dW_f}{dg'_f} \\ \frac{dW_i}{dg'_i} &= c_m \frac{\partial W_m}{\partial m_i} \frac{dm_i}{dg'_m} + c_m \frac{\partial W_m}{\partial m} \frac{dm}{dg'_m} \\ &\quad + c_f \frac{\partial W_f}{\partial m_i} \frac{dm_i}{dg'_f} + c_f \frac{\partial W_f}{\partial m} \frac{dm}{dg'_f} . \end{aligned} \quad (2.2)$$

The terms dm_i/dg'_m and dm_i/dg'_f represent the correlations between the genotypes of males and females produced in nest i and the strategy m_i applied by workers of that nest. The two ratios correspond to worker-offspring relatedness r_m and r_f . Similarly, dm/dg'_m and dm/dg'_f express the correlations of descendant genotypes with the average strategy in the population, m , corresponding to the relatedness between male and female offspring of the focal colony and a random member of the population, R_m and R_f . We assume that the population is very large and random mating occurs. Therefore, R_m and R_f are equal to zero and terms in Equation 2.2 containing dm/dg'_m and dm/dg'_f cancel.

Substituting the expressions for W_m and W_f into Equation 2.2 we obtain

$$\begin{aligned} \frac{dW_i}{dg'_i} &= c_m r_m \left(-\frac{s'_i}{1-s} (1 - \gamma_i) - \frac{1 - s_i}{1-s} \gamma'_i \right) \\ &\quad + c_f r_f \left(\frac{s'_i}{s} (1 - \gamma_i) - \frac{s_i}{s} \gamma'_i \right) , \end{aligned} \quad (2.3)$$

where s' and γ' denote derivatives of s and γ with respect to m_i . Solving $\partial W_i / \partial g'_i = 0$ at $m_i = m$ gives us the equilibrium condition for sex ratio manipulation

$$\gamma' (c_m r_m + c_f r_f) = s' (1 - \gamma) \left(c_f r_f \frac{1}{s} - c_m r_m \frac{1}{1 - s} \right). \quad (2.4)$$

Equation 2.4 is arranged so as to emphasise the selective forces acting on worker sex ratio manipulation. The left-hand side corresponds to the force of selection acting on the colony-level against manipulation of sex allocation. It contains the change in colony productivity (γ') caused by a change in worker strategy m . The right-hand side assembles the benefit of sex ratio manipulation which is mainly determined by the difference in the workers' relatedness to female and male sexuals.

In the case where sex ratio manipulation has no cost ($\gamma(m_i) = 0$), colony level selection is null ($\gamma' = 0$). If we solve for the evolutionary stable (ES) manipulation m^* while substituting $s = p + m$, we recover Trivers and Hare's prediction for sex allocation under worker control as

$$m^* = \frac{c_f r_f}{c_f r_f + c_m r_m} - p. \quad (2.5)$$

In the absence of cost, workers will manipulate sex allocation up to a value of $c_f r_f / (c_f r_f + c_m r_m)$, i.e. up to a secondary sex ratio of 0.75 in the case of a colony headed by a single once-mated queen.

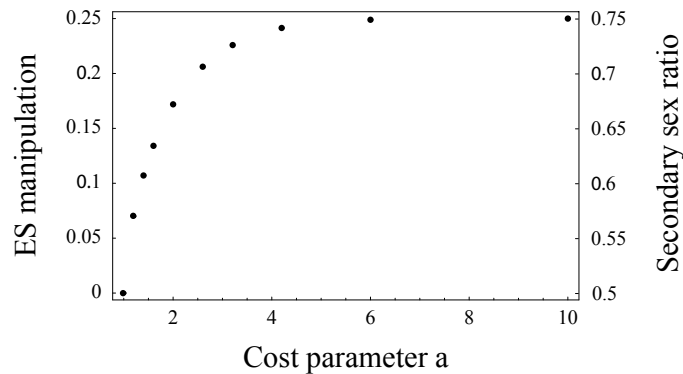


Figure 2.2: ES sex ratio manipulation by workers (m^*) and the resulting colony sex ratio (s^*) as a function of the cost of manipulation (a). Calculations assume $c_f r_f = 0.75$, $c_m r_m = 0.25$, and $p = 0.5$.

With costs of manipulation, however, the reduction in colony productivity selects against sex ratio manipulation and the workers' optimal sex allocation departs from the 3:1 ratio predicted by Trivers and Hare. The extent of deviation depends on the steepness of the cost function γ , i.e. the value of a (Fig. 2.2). If cost is a linear function of manipulation ($a = 1$), workers are not selected to manipulate sex ratio at all and workers rear brood in the sex ratio produced by queens ($s = p$, Fig. 2.2). With the cost functions becoming flatter (increasing values of a), ES manipulation increases and sex allocation becomes more female-biased. For values of a becoming very large, ES sex ratio manipulation asymptotically approaches $m = 0.25$, corresponding to the Trivers and Hare prediction of a 3:1 sex ratio ($s = 0.75$).

So far we have assumed that the cost of sex ratio manipulation is equal in all colonies of a population. However, the cost of eliminating brood or influencing female cast might depend on environmental factors, such as resource availability or micro-climate. Spatial variations in these factors are therefore expected to lead to variations in cost of sex ratio manipulation. Extending Equation 2.1, we now introduce such variations in our model. We analyse the simplest case possible, consisting of two classes of colonies that differ in the form of the cost function γ , i.e. in the value of parameter a . Colonies of the two classes occur with constant frequencies q_1 and $q_2 = 1 - q_1$. We assume that class membership is determined by the environment and independent of genotype. Workers can detect which class their colony belongs to and in response apply one of two sex ratio manipulation strategies. The two strategies evolve as independent characters, but sex allocation in the two classes is linked because sexuals from both classes compete with each other for matings.

We denote class with subscript j . Workers in colony i of class j manipulate sex ratio by m_{ji} resulting in a secondary sex ratio $s_{ji} = p + m_{ji}$, where p is again assumed to be constant over colonies and classes. Manipulation reduces colony productivity by $\gamma(m_{ji}) = \gamma_{ji} = m_{ji}^{a_j}$. Class means of manipulation, secondary sex ratio, and cost are denoted by m_j , s_j , and γ_j , respectively. As before, the evolutionary change of sex ratio manipulation is given by the derivative of fitness with respect to offspring genotype

$$\begin{aligned} \frac{dW_{ji}}{dg'} &= c_m r_m \left(-\frac{s'_{ji}}{1-s} (1-\gamma_{ji}) - \frac{1-s_{ji}}{1-s} \gamma'_{ji} \right) \\ &\quad + c_f r_f \left(\frac{s'_{ji}}{s} (1-\gamma_{ji}) - \frac{s_{ji}}{s} \gamma'_{ji} \right). \end{aligned} \quad (2.6)$$

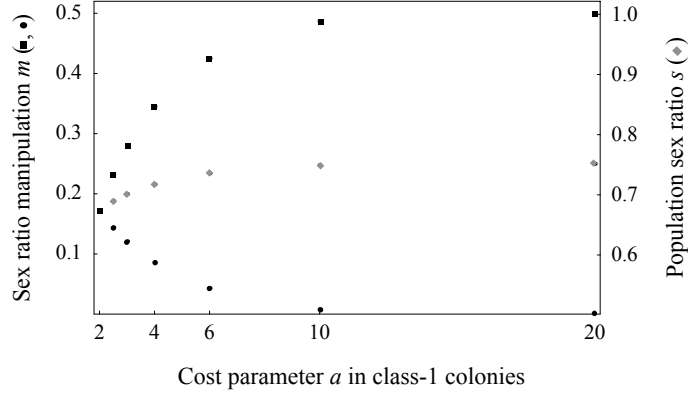


Figure 2.3: ES sex ratio manipulation by workers in the two colony classes (m) and population sex ratio (s) as a function of cost of manipulation (a) in class 1. Values of m for class 1 are given as black squares, those for class 2 as black circles. Population sex ratio is given as grey diamonds. Cost in class 2 is constant ($a = 2$) and calculations assume $q_1 = q_2 = 0.5$, $c_f r_f = 0.75$, $c_m r_m = 0.25$, and $p = 0.5$ for both classes.

where

$$s = \sum q_j \frac{1 - \gamma_j}{1 - \sum \gamma_j} s_j \quad (2.7)$$

is the population sex ratio with class sex ratios being weighted by class frequencies and relative productivities.

The equilibrium level of sex ratio manipulation has to be calculated for the two classes separately by setting $\partial W_{ji} / \partial g'_{ji} = 0$ and solving at $s_{ji} = s_j = s_j^*$. Sex ratio manipulation in class j is at equilibrium when

$$\gamma'_j \left(c_m r_m \frac{1 - s_j^*}{1 - s} + c_f r_f \frac{s_j^*}{s} \right) = s'_j (1 - \gamma_j) \left(c_f r_f \frac{1}{s} - c_m r_m \frac{1}{1 - s} \right) \quad (2.8)$$

Figure 2.3 shows ES levels of sex ratio manipulation in two equiproportional classes ($q_1 = q_2 = 0.5$). As expected, equal cost in both classes ($a_1 = a_2 = 2$) leads to a unique ES strategy and workers in all colonies manipulate sex ratio to the same degree. However, if cost varies between colony classes, equilibrium strategies diverge. Colonies in the class with cheaper manipulation (low-cost colonies) will bias sex ratio to a greater degree than colonies in which manipulation is more costly (high-cost colonies). The divergence between the

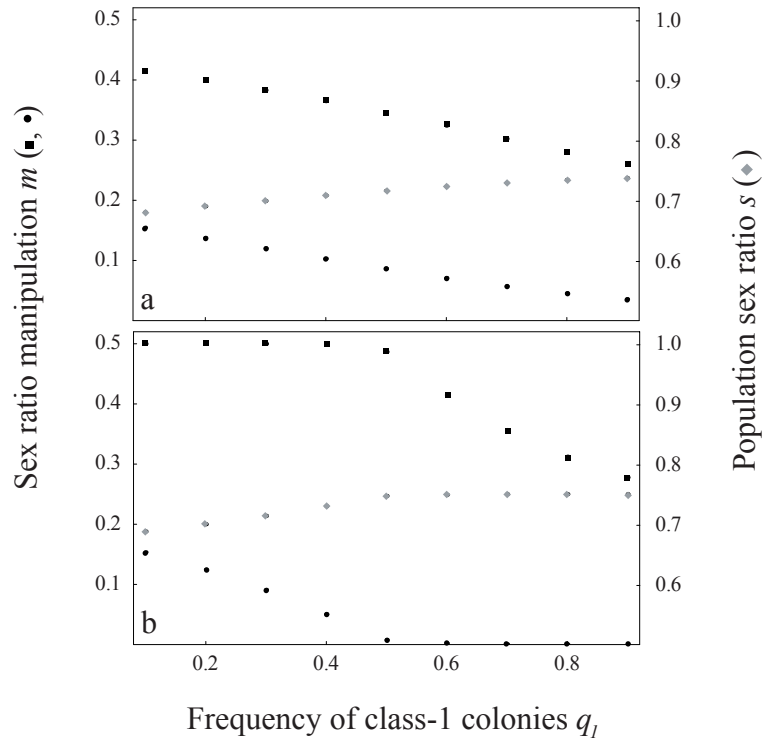


Figure 2.4: ES manipulation by workers in the two colony classes (m) and population sex ratio (s) as a function of the frequency of class 1 (q_1). ES manipulation in classes 1 and 2 are given as black squares and circles, respectively, population sex ratio as grey diamonds. Calculations assume $q_1 = q_2 = 0.5$, $c_{frf} = 0.75$, $c_{frf} = 0.25$, and $p = 0.5$ for both classes. a) $a_1 = 4, a_2 = 2$. b) $a_1 = 10, a_2 = 2$

two ES strategies exceeds that expected based on the difference in cost alone (cf. Fig. 2.2). The reason is that competition in the mating population drives investment strategies of the two classes apart. The female bias in the sexuals produced by low-cost colonies decreases female mating success, which in turn selects workers in high-cost colonies to reduce sex ratio manipulation. How much sex ratio manipulation diverges between the two classes depends on how different their cost functions are. As shown in Figure 2.3, decreasing cost in one class (i.e. increasing a) while leaving it constant in the other class results in stronger sex ratio specialisation in both classes. Along with this goes an increase in overall population sex ratio, caused by the reduction in mean cost in the population (Fig. 2.3, diamonds).

The ES levels of sex ratio manipulation also depend on the frequencies of

the two colony classes in the population. As illustrated in Figure 2.4, the strategy of workers in a given class becomes extremer as that class gets rarer. This can lead to no manipulation ($m = 0$) or complete manipulation ($m = 0.5$) with low frequencies of high- and low-cost colonies, respectively (Fig. 2.4b). Class frequencies also have an influence on population sex ratio, which becomes more female biased as the frequency of low-cost colonies increases (Fig. 2.4, diamonds). However, workers in low-cost colonies will prevent an increase of the population female bias beyond the worker optimum of $s = 0.75$ by adapting their level of sex ratio manipulation (Fig. 2.4b).

Discussion

The models presented in this article demonstrate the importance of proximate mechanisms for the evolution of optimal sex allocation under worker control. Because workers have to manipulate the brood sex ratio in order to adjust sex allocation, the negative effects of this manipulation on colony productivity will select against sex ratio biasing by workers. The impact of costs of sex ratio manipulation on the evolution of sex allocation is therefore opposed to that of relatedness asymmetry (RA), which selects workers for biasing colony sex allocation towards more closely related females. Under the influence of these two opposing forces, sex allocation will evolve towards an equilibrium at which the fitness gain of biasing sex ratio towards females is equal to the fitness loss due to the reduction in overall production of sexuals.

By selecting against sex ratio biasing, costs of brood manipulation can significantly decrease female-bias in the workers' optimal sex allocation. For example, a cost that would reduce colony productivity by less than 5% in order to attain the Trivers and Hare sex ratio of 3:1 (cost parameter $a = 2.2$) shifts the worker optimum from 3:1 to 2.2:1. The strong effect of costs on the workers's sex ratio optimum might partly account for the global deviation of sex ratios produced by monogynous ant species from the 3:1 ratio predicted by Trivers and Hare (Pamilo, 1990; Boomsma, 1989). So far, two explanations had been invoked in order to explain this discrepancy. First, social structure in the species classified as monogynous might deviate in some way from the assumptions of the Trivers and Hare model (Bourke and Franks, 1995; Boomsma, 1989; Nonacs, 1986). For example, polygyny (multiple queen colonies) with related queens, multiple mating by queens or worker reproduction might occur at low frequencies. All of these lower RA and therefore decrease female bias

in the workers' equilibrium sex ratio. Alternatively, workers and queens might share control in some of the species. Colony sex allocation is then expected to be intermediate between the workers' 3:1 and the queen's 1:1 optima (e.g. Matessi and Eshel, 1992, Chapter 3). However, costs of sex ratio manipulation perhaps provide a more general explanation for why sex allocation falls short of the 3:1 prediction. The egg sex ratio being naturally controlled by the queen, worker control of sex allocation requires the manipulation of brood. Whenever this manipulation is associated with costs (which is probably true in most cases) we expect sex allocation to be less female-biased than 3:1.

The extent to which optimal sex allocation deviates from the value predicted by RA depends on the magnitude of costs: the more costly sex ratio manipulation, the greater the deviation. Variations in cost between species, between populations, or between colonies might therefore account for the variations in sex allocation that have been observed at these different scales (e.g. Pamilo, 1990; Brian, 1979; Brown and Keller, 2000). The exact causes for the variations in cost may differ between the levels of organisation. Between species, differences in cost are most likely due to the exact mechanisms used by workers to manipulate sex ratio. Species with more costly mechanisms of sex ratio biasing are expected to have a less female-biased sex allocation than species in which sex ratio manipulation is more efficient. Adjusting the brood sex ratio by eliminating male brood, for example, might affect colony productivity differently than adjusting brood sex ratio by biasing female caste determination. Morphological differences between taxa might also influence the cost of sex ratio biasing. If workers use the size of larvae and pupae as a cue to determine the sex of the brood, they are expected to have more difficulties in discriminating between males and females in species where both sexes are of similar size. This, in turn, should increase the cost of sex ratio manipulation due to the risk of accidentally manipulating brood of the wrong sex. Consequently, we predict a global correlation between sexual dimorphism and female bias (Chapter 3). This trend has indeed been described, albeit with a different interpretation (Boomsma, 1989).

At the level of populations or colonies, variations in cost are more likely to result from environmental factors. For example, climatic conditions may influence whether females raised in replacement of eliminated males are able to complete development before the end of the reproductive season. Similarly, food availability might determine to what extent the loss of resources due to the elimination of males affects colony productivity. An association between resource availability and population sex ratio has indeed been reported from

the ant species *Tetramorium caespitum* (Brian, 1979), *Lasius niger* (Boomsma *et al.*, 1982), *Myrmica sulcinodis* (Elmes, 1987), and *Leptothorax longispinosus* (Herbers, 1990). However, in *L. niger* and *L. longispinosus* the variations in sex allocation could be shown to be independent of habitat quality (van der Have *et al.*, 1988; Backus and Herbers, 1992). It is therefore not clear whether resource availability generally affects costs of brood manipulation and sex allocation and whether the effects of resources on sex allocation can be overridden by other factors.

Our models have shown that variations in cost of sex ratio manipulation between colonies are amplified by competition in the mating population and result in even stronger variations in sex allocation. Colonies in which costs are high will produce relatively less females than expected on the basis of costs alone, while colonies where costs are low produce relatively more females. Sex ratio specialisation (or split sex ratios) have been predicted to arise in two other situations. Grafen (1986) argued that sex ratio specialisation is expected to occur whenever the relative cost of producing males and females differs between colonies. Colonies will then invest more in the sex which, relative to the population mean, is cheaper for them to produce. Alternatively, split sex ratios have been predicted to arise from variations in RA between colonies which create differences in the relative benefit of producing males and females (Boomsma and Grafen, 1990, 1991). If colonies vary in their social structure, then workers in colonies in which RA is above the population average will favour a higher investment in females. Workers in colonies with below-average RA will favour to produce more males. The association of RA with sex ratio specialisation has been investigated empirically in many species exhibiting split sex ratios. While in some cases the observed variation in colony sex allocation could effectively be attributed to variations in RA, in many other species it could not (e.g. Helms, 1999; Brown and Keller, 2000; Fjerdingstad *et al.*, 2002). In these cases, the consideration of proximate factors such as costs of sex ratio manipulation or male and female production might explain some of the observed variance in sex allocation.

In conclusion, this work demonstrates that proximate mechanisms can account for some of the deviations of empirical sex ratio data from predictions based on ultimate arguments alone. Presently, it is impossible to test our model because we lack information on costs of sex ratio manipulation in natural colonies of social insects. Obtaining these data is difficult because the level of sex ratio biasing has to be manipulated indirectly. This could be done by artificially changing the primary sex ratio. Increasing the proportion of fe-

males among sexual brood approaches the sex ratio to the workers' optimum and should reduce the level of manipulation, while increasing the proportion of males should have the opposite effect. Measuring productivity in colonies with manipulated brood sex ratio should allow to assess the costs of sex ratio manipulation. Such data, although difficult to obtain, would finally allow us to complete the circle from ultimate causes of queen-worker conflict to the proximate mechanisms of worker sex ratio manipulation and back to their ultimate impact on the worker sex ratio optimum.

Summary

In eusocial Hymenoptera, queens and workers are in conflict over optimal sex allocation. Sex ratio theory, while providing us with predictions on the extent of conflict under a wide range of conditions, has neglected the fact that worker control of investment requires the manipulation of brood sex ratio. This manipulation is likely to incur costs, for example if workers eliminate male eggs to bias sex allocation towards more females. In this paper, we present a model of sex ratio evolution under worker control that incorporate costs of brood manipulation. We assume costs to be a continuous, increasing function of the magnitude of sex ratio manipulation. We demonstrate that costs exert a selective force at the colony level that counter-acts individual selection in favour of sex ratio biasing. Thus, costs of sex ratio manipulation can account for part of the discrepancies between theoretical and observed values of sex allocation. Further, we show that variations in costs of sex ratio manipulation between colonies can lead to split sex ratios. This suggests that costs of manipulation might be a factor that, in addition to variation in relatedness asymmetry and heterogeneity in resource availability, induces sex ratio specialisation.

Acknowledgements

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Chapter 3

Sex ratio conflict and worker production in eusocial Hymenoptera

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Introduction

One of the major trade-offs in an organism's life-history is that between investment in reproduction versus body growth (Stearns, 1992). Spending resources on immediate reproduction provides direct fitness gain but decreases somatic growth and expected future reproduction. The evolutionary optimal strategy is continued growth until the fitness gain due to increased size is exactly counterbalanced by the fitness obtained by immediate reproduction. This principle can be applied to predict investment in colony growth in social insects with morphological castes, whereby workers are the equivalent of somatic tissue and new queens (gynes) and males constitute the reproductive investment. However, in contrast to unitary organisms which consist of clonal cells ($r = 1$), colonies of social insects contain individuals whose relatedness is lower than one ($r < 1$). This genetic heterogeneity results in potential genetic conflicts over resource allocation and reproductive decisions (Hamilton, 1964; Pamilo, 1991; Ratnieks and Reeve, 1992; Keller and Chapuisat, 1999; Keller and Reeve, 1999). Genetic conflicts are of particular importance in eusocial Hymenoptera (ants, bees and wasps), whereby the haplodiploid sex-determination system creates relatedness asymmetries among family members. Because workers are more closely related to their sisters than to their brothers they should favor a female-biased sex allocation (here defined as the relative investment in gynes and males). In contrast, queens are equally related to their sons and daughters and favor an even resource investment into both sexes. Thus, queen-worker conflict over sex-allocation arises.

Sex ratio conflict has been extensively investigated both theoretically and empirically (e.g. Trivers and Hare, 1976; Charnov, 1978; Nonacs, 1986; Boomsma and Grafen, 1990; Boomsma *et al.*, 1999; Pamilo, 1991; Sundström, 1994; Bourke and Franks, 1995; Crozier and Pamilo, 1996; Chapuisat and Keller, 1999). In contrast, little attention has been paid to the question of how conflict among colony members extends to resource allocation to colony growth and maintenance (worker production) versus reproduction (production of males and gynes), and contradictory conclusions have been reached by different authors. Pamilo (1991) suggested that conflict over colony growth arises between queens and workers, with workers preferring a greater investment in gynes as opposed to workers than the queen. However, Bourke and Chan (1999) suggested that conflict between queens and workers should be absent if the relative investment in worker and sexual production does not affect a colony's sex allocation (the proportional investment in gynes versus males). That is, both

queens and workers should favor the investment in workers that ultimately maximises the lifetime colony production of reproductive individuals. Finally, yet another conclusion was reached by Bourke and Ratnieks (1999) while investigating conflict over caste determination, the irreversible process during which a female larva morphologically and physiologically specialises to become either a worker or a new queen. The authors found conflict between queens and workers to arise over the developmental pathway of individual female larvae, with workers preferring a higher proportion of females larvae to develop into gynes than the queen. The analysis of Bourke and Ratnieks (1999) also included the interests of the female brood itself and they found that female larvae are in conflict with queens and workers under a wide range of conditions. Because female larvae are more closely related to themselves than to the sexuals they would raise as workers they frequently benefit to become sexuals under conditions where queens and workers would benefit from increasing the size of the colony's workforce.

In this paper we reconsider the problem of potential conflict over resource allocation in the production of workers, males, and gynes. We construct a simple and general kin-selection model which allows to investigate the simultaneous evolution of investment in workers and sex allocation. This is a significant step towards a better understanding of optimal reproductive allocation and sex ratio conflict because previous models dealt either with sex allocation, neglecting investment in colony growth, or with optimal investment in colony growth in the situation where sex allocation is fixed. Moreover, in our analyses, we explicitly consider the power that queens and workers have to manipulate resource allocation and the selection pressures acting on queens, workers and larvae to do so.

Our model shows that queen-worker conflict occurs over sex allocation but not colony growth. The evolutionary stable investment in workers is the same for both parties when one of them has complete control over resource allocation, i.e., the relative investment in workers, gynes, and males. Complete control by one party is, however, evolutionary unstable because it strongly selects the other party to manipulate sex allocation in its favor. When no party can monopolise control over resource allocation, the investment in worker production is predicted to be lower than the two parties' optimum. This is because the queen's and workers' attempts to bias sex allocation in their favor are at the detriment of worker production. Our model also confirms the occurrence of conflict between adult colony members and female larvae over their developmental fate. Under most conditions individual female larvae benefit to

escape adult control of caste determination and develop into gynes. Finally, our model sheds light on the reasons for which previous authors had reached different conclusions about whether or not queens and workers are in conflict over the relative amount of resources that should be allocated to worker production.

The Model

In our model, we consider a colony with one or several queens (mated with a variable number of males) and sterile workers. Males and gynes are assumed to disperse so that neither inbreeding, nor local mate competition (Hamilton, 1967), nor local resource competition (Clark, 1978) occur. Workers, gynes and males are assumed to be equally costly to produce. Therefore, the proportional investment into one class of individuals corresponds to the number of individuals of this class relative to the total number of individuals produced. We will thus indiscriminately use sex ratio and sex allocation throughout the manuscript. In the colony, investment in workers, gynes, and males is determined by two parameters. The first one, f , describes the proportion of resources that are allocated to the production of female, i.e., workers and gynes (hereafter referred to as proportional investment in females). Correspondingly, the quantity $(1 - f)$ is the proportion of resources invested in the production of males. The second parameter determining resource allocation is w , the proportion of the allocation to females that is devoted to the production of workers. The remainder $(1 - w)$ goes into the production of gynes. Because of the equal cost of gynes and workers, the parameter w therefore describes the proportion of females that will develop into workers. In sum, the colony invests a proportion fw of resources in workers, a proportion $f(1 - w)$ in gynes, and $(1 - f)$ in males. The population means of the proportional investments are given by capital letters, i.e., FW into workers, $F(1 - W)$ into gynes, and $(1 - F)$ into males. For the sake of simplicity, our model does not consider any temporal variation of investment that might occur in the course of colony ontogeny. It predicts the overall investment in workers, gynes, and males over the whole colony life.

Because per worker productivity declines with the number of workers per colony in eusocial Hymenoptera (Brian, 1956; Tschinkel, 1993; Sundström, 1995), overall colony productivity (biomass of workers, gynes, and males produced) is assumed to increase with colony size according to the following di-

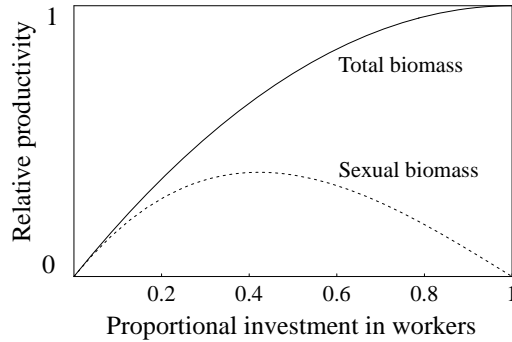


Figure 3.1: Relative productivity of a colony as a function of proportional investment in workers. The solid line shows the total productivity (workers and sexuals), the dotted line the productivity of sexuals only.

minishing return function $b(f, w) = 1 - (1 - fw)^2$. Under this function, productivity varies between zero when the relative investment in workers is zero and 1 (maximum productivity), when all resources are allocated to worker production (Figure 3.1, solid line). The productivity in terms of sexual biomass (gynes and males) is given by $b(f, w)(1 - fw)$ (Figure 3.1, dotted line). (Note that choosing another functions with diminishing returns does not affect the qualitative results of our model.)

The inclusive fitness V_X of a colony member X depends on the biomass of males and gynes produced, the regression relatedness of females (g_{fX}), and males (g_{mX}) to X , the reproductive values of females ($\nu_f = 1$) and males ($\nu_m = 0.5$), and the mating success of female reproductives ($1/[F(1 - W)]$) and males ($1/[1 - F]$) (Crozier and Pamilo, 1996). Taking into account these variables, the fitness of individual X is given as

$$V_X = b(f, w) \left(\frac{f(1 - w)g_{fX}\nu_f}{F(1 - W)} + \frac{(1 - f)g_{mX}\nu_m}{(1 - F)} \right) \quad (3.1)$$

The fitness function (Eq. 3.1) allows us to calculate the evolutionary stable (ES) values for the proportional investment in females f and the proportion of females developing into workers w in function of (i) who in the colony has control of these two variables, and (ii) the kin structure of the colony.

The question of who controls the two parameters (f and w) that determine resource allocation is important because queens and workers might differ in their optimal values. The queen can produce males by laying unfertilised

haploid eggs and females by producing fertilised diploid eggs. She is therefore a priori in control of proportional investment in females. However, workers may alter the sex allocation by selectively eliminating brood of one sex [most likely males, as has been demonstrated in the ant *Formica exsecta* (Sundström *et al.*, 1996; Chapuisat *et al.*, 1997)] to raise more individuals of the other sex. In this way workers can gain control over proportional investment in females.

There has been considerable discussion about who (the queen, the workers or the female larvae) is in control of caste determination, and therefore able to regulate the proportion of females that develop into workers versus gynes. Female development had long been assumed manipulated by queen produced pheromones (Brian, 1980; Fletcher and Ross, 1985; Hölldobler and Bartz, 1985). However, this view has been challenged because there is no empirical data in favor of pheromonal control, whereby queens are able to chemically force female larvae to develop into workers rather than queens (Keller and Nonacs, 1993). In fact, there is increasing evidence that queen pheromones act as honest signals of queen fecundity to which other colony members respond by altering reproductive decisions (e.g. Pettis *et al.*, 1997; Ortius and Heinze, 1999). An alternative way for queens to influence caste determination is by producing different types of eggs, which has been documented in two species of ants. In *Pheidole pallidula* queens produce diploid eggs that vary in their content of juvenile hormone and develop either into workers or gynes (Passera, 1980; Wheeler, 1986). Similarly, *Formica polyctena* queens can apparently bias caste determination by varying eggs size and the amount of RNA and other biochemical substances in the eggs (Wheeler, 1986, and references therein). Since it is yet unclear who is controlling caste determination and because there might be interspecific variation, we will consider the three possible scenarios of caste determination, with the developmental fate of larvae being either under the control of the queen, the workers or the larva itself.

In our model we will first consider the case where either the queens or workers have complete control over f and w , i.e., the proportional investment in females and the proportion of females raised as workers. Complete queen control may occur when (i) queens limit the number of eggs produced thus making eggs a limiting resources which forces workers to raise all the brood present in the colony (Rosenheim *et al.*, 1996), and (ii) queens control caste determination, which may be the case in species with blastogenic caste determination (i.e., when maternal effects determine the developmental fate of female brood; Passera, 1980; Wheeler, 1986). Alternatively, complete worker

control may occur when i) the number of female eggs is not limited, ii) workers are able to assess the sex of eggs or young larvae and eliminate males without any cost and thereby alter the queen-produced primary sex ratio, and iii) workers control caste determination, for example through differential feeding of the larvae.

Next we will consider the case of mixed queen and worker control over resource allocation. Although single-party control is possible under a restricted set of conditions (see above) queens and workers most likely share control over investment in the majority of species (Trivers and Hare, 1976; Bourke and Franks, 1995). This is because when sex allocation is at one party's optimum, the other party is under strong selection to manipulate sex allocation to its own advantage. We will consider the most likely situation of mixed control, that is queens control proportional investment into females (workers + gynes) by limiting the number of eggs, whereas workers control caste determination, that is the proportion of females developing into workers versus gynes. Note that in this case worker fitness will be principally limited by the number of female eggs available and not so much the overall number of eggs. Active egg limitation by the queen is therefore equivalent to a limitation of female eggs only, in which case workers raise all female eggs and invest the remaining resources in the rearing of as many male eggs as possible.

Single-party control

The ES investment pattern when a single party is in control of resource allocation (f and w) can be found by substituting the corresponding values of relatedness for that party in Eq. 3.1 and maximizing the function simultaneously for f and w at $w = W = w^*$ and $f = F = f^*$. For queen control under monogyny (single-queen colonies) and monandry (single queen mating) we substitute the relatedness values $g_f = 0.5$ and $g_m = 1$. The ES solution is ($f_Q^* = 0.71, w_Q^* = 0.59$), meaning that 71% of the resources are invested in females of which 59% go into workers. The resulting allocation pattern is 42% of the resources invested in workers, 29% in gynes, and 29% in males. The investment in males and gynes is even, as predicted from sex ratio theory when queens control colony sex allocation (Trivers and Hare 1976), and the allocation to workers is the one that maximises sexual production (cf. Fig. 3.1). The numerical value of the relative investment in workers has of course no biological meaning as it critically depends on the function $b(f, w)$ that was chosen. A different ES investment in workers would be found if one would use

a different function, but the important point is that it would always be the one that maximises the overall productivity of males and gynes.

The ES values of f and w under complete worker control of resource allocation can be obtained in the same manner by substituting $g_f = 0.75$ and $g_m = 0.5$ in Equation 1 and maximizing for f and w . The values obtained are ($f_W^* = 0.86$, $w_W^* = 0.49$), which corresponds to an investment of 42% in workers, 44% in gynes, and 14% in males. The optimal investment in workers is the same as for queens and it is the value which maximises the colony's overall production of gynes and males. However, contrary to queens, workers favour a three times higher investment in gynes than males, as predicted by sex ratio theory (Trivers and Hare, 1976). [Note that the small deviation of the predicted sex investment ratio (44% gynes : 14% males) from the theoretical prediction of 3:1 is due to rounding].

More complex kin structures that result from polyandry (multiple mating by queens) or polygyny (multiple reproductive queens per colony) affect relatedness asymmetries between colony members and thus the workers' optimal sex allocation. However, both, under complete queen and complete worker control, the ES investment in workers is not influenced by the level of polyandry or polygyny, because both parties benefit from maximizing overall colony productivity of sexuals.

In summary, complete control of resource allocation by either queens or workers leads to each party favouring the relative investment in worker production that maximises overall productivity of sexuals, and this is true whatever the kin structure of the colony. In contrast, queens and workers diverge in the optimal investment in males and gynes, as expected from sex ratio theory. Consequently, queen-worker conflict arises over the allocation of resources to male and female reproductives not but not over worker production.

Mixed control

When colony and population sex allocation are at one party's optimum, this exerts a selective pressure on the other party to bias sex allocation to its own advantage. Our model allows to determine the strength and direction of this selective pressure by calculating the selection gradient, which is the coefficient of a regression of fitness on phenotype (Lande and Arnold, 1983). The selection gradient is equivalent to the derivative of the fitness function with respect to phenotype. We can thus quantify the selection acting on queens and workers

by calculating the partial derivatives of their inclusive fitnesses with respect to f and w .

Consider first the case of complete worker control under monogyny and monandry. The predicted investment in workers is 42% and the sex allocation 3:1 female biased. The gradient of selection on queens to alter the proportional investment in females is given by the partial derivative of Equation 3.1 with respect to f . Substituting the values $g_f = 0.5$, $g_m = 1$, $F = 0.86$, and $w = W = 0.49$ gives a partial derivative of $\beta_f = \partial V_Q / \partial f = -1.35$. This negative value indicates that complete worker control of resource allocation selects queens to decrease the colony's proportional investment in females, which can be achieved by producing a more male biased primary sex ratio and limiting the number of eggs laid.

Alternatively, in the case of complete queen control of caste allocation, the investment in workers still is 42%, but the sex allocation is now even. The gradient of selection acting on workers can be obtained by the partial derivative of worker fitness with respect to w at $f = F = 0.71$, $W = 0.59$, and substituting the workers' relatedness to males and gynes ($g_f = 0.75$, $g_m = 0.5$), which gives $\beta_w = \partial V_W / \partial w = -0.41$. This negative value indicates that workers benefit from directing a greater proportion of the female brood towards gyne development even though this decreases overall colony productivity.

In summary, neither complete queen control nor complete worker control of resource allocation are stable evolutionary equilibria. Both situations lead to strong selection on the party not in control of resource allocation to manipulate investment to its own favour. If manipulation is possible, mixed control of caste allocation will arise and queen and worker strategies of manipulation and counter-manipulation will evolve in response to each other. A stable equilibrium will be reached when (i) the queen cannot increase her inclusive fitness by altering the proportional investment in females via the primary sex ratio produced, and (ii) workers cannot increase their inclusive fitness by changing the proportion of female larvae raised as workers versus gynes. We can find the ES equilibrium for f and w by solving the equation system

$$\begin{aligned} \frac{\partial V_Q}{\partial f} \Big|_{f=F=f^*} &= 0 \\ \frac{\partial V_W}{\partial w} \Big|_{w=W=w^*} &= 0 \end{aligned} \quad (3.2)$$

while substituting the corresponding relatedness values. Under monandry and

monogyny the equilibrium values are $f^* = 0.72$ and $w^* = 0.51$, corresponding to an investment of 36% in workers, 35% in gynes, and 29% in males. This indicates that colony productivity is suboptimal, because the investment in workers is smaller than 42%. Sex allocation is intermediate between the queen and worker optima, the female-to-male investment ratio being at 1.2 : 1. (Note again that the numerical values should only be considered as indicative of the qualitative, but not quantitative change in caste allocation.)

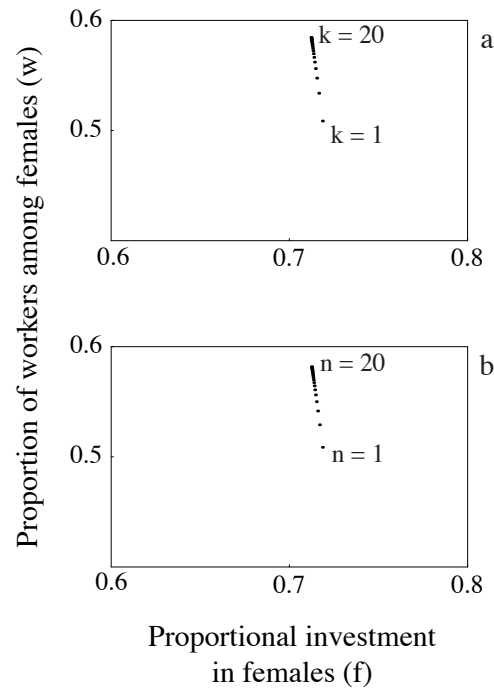


Figure 3.2: Evolutionary equilibria of proportional investment in females (f), and proportion of workers among females (w), as a function of a) queen mating frequency (k) (assuming monogyny and equal paternity among males), and b) the number of nestmate queens (n) [assuming full sister queens ($g_{QQ} = 0.75$), single mating, and equal contribution of queens to the production of workers, males, and gynes]. f is assumed to be under queen control whereas w is controlled by the workers.

The ES values of f and w under polyandry or polygyny can be calculated by substituting the corresponding relatedness values in Equation 3.2. Polyandry reduces the relatedness among female offspring of the queen (workers and gy-

nes) whereas worker-male relatedness remains constant. Hence, relatedness asymmetry decreases as queen mating frequency increases and workers benefit less from biasing sex allocation toward females. Therefore, multiple mating reduces conflict between queens and workers over sex allocation and ES resource allocation tends towards the values obtained under complete queen control of resource allocation (Fig. 3.2a, calculations assume even paternity of the queen's mates). Concurrently with this tendency, higher queen mating frequency results in an increase in the proportional investment in workers which asymptotically approaches the optimal value of 42% (Fig. 3.3a).

Increased levels of polygyny also tend to lead to a less females-biased sex allocation and to an increase in the relative investment in workers (Figs. 3.2b and 3.3b, calculations assume equal contribution of queens to the production of workers, gynes, and males). The effect of increased queen number is the stronger the more closely queens are related. If queens are unrelated, the ES sex allocation and the relative investment into workers are not affected by changes in queen number and remain the same as under monandry and monogyny. This is because relatedness asymmetry is unaffected by variation in queen number when queens are unrelated (Frank, 1987).

Larval control of caste determination

So far we have only considered the workers' and queens' interests. We will now investigate whether the interests of the developing larvae diverge from those of workers and queens. Diverging interests of female larvae would be indicated by selection favoured larvae escaping adult control of caste determination. Whether this is the case can be investigated with our model. Consider a mutant larva that controls its own caste determination and develops with probability ω into a worker and $(1 - \omega)$ into a gyne. Other larvae in the colony in which the mutant occurred become workers and gynes with probabilities w and $(1 - w)$, respectively, whereby w is determined by either the queen or workers. Assume also, for simplicity, that the population investment patterns are the same as in this colony, i.e. $W = w$ and $F = f$.

The inclusive fitness of the mutant larvae V_M is equal to the sum of its inclusive fitness if developing into a worker (ΔV_W) and the inclusive fitness if developing into a gyne (ΔV_G), weighted by the respective probabilities, $V_M = \omega(\Delta V_W) + (1 - \omega)(\Delta V_G)$. The inclusive fitness gained by worker development ΔV_W is equal to the number of additional males and gynes a colony would produce with the help of one additional worker multiplied by the larva's

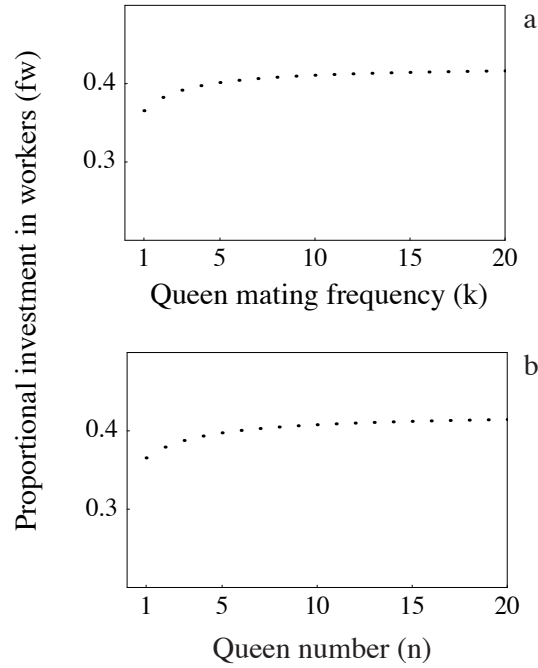


Figure 3.3: Evolutionary equilibrium of proportional investment in workers (fw) in the case where queens control the proportional investment in females (f) and workers control the proportion of workers among females (w), as a function of a) queen mating frequency (k) (assuming single-queen colonies and equal paternity among males), and b) the number of nestmate queens (n) [assuming full sister queens ($g_{QQ} = 0.75$), single mating, and equal contribution of queens to the production of workers, males, and gynes].

relatedness to these sexuals and their mating success. The increase in colony productivity can be calculated as follows. If $s(f, w) = b(f, w)(1 - fw)$ describes the relative production of sexuals in the focal colony, the number of extra sexuals produced due to one additional worker is given by $\partial s / f \partial w$. This expression is the slope of sexual production on investment in workers (fw). The division by f is necessary because overall colony productivity varies between zero and one, whereas the investment in workers (fw) varies between zero (for $w = 0$) and f (for $w = 1$). Dividing by f corrects for this difference in scale and one obtains the number of sexuals produced per worker. The direct fitness obtained by a larva developing into a gyne (ΔV_G) is simply equal to her expected mating success [$1/F(1 - W)$], its relatedness to itself being one. The overall mutant inclusive fitness can thus be written as

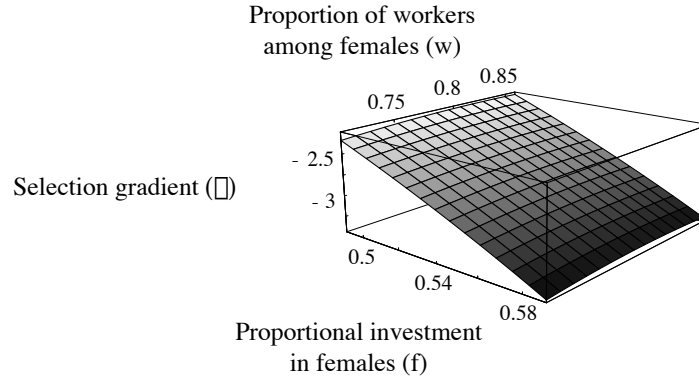


Figure 3.4: Selection differential (β) on a mutant female larva's probability to develop into a worker (ω) as a function of egg sex ratio (f) and proportion of female larvae raised as workers (w).

$$\begin{aligned}
 V_M = & \omega \frac{\partial s}{\partial w} \frac{1}{f} \left[\frac{f(1-w)}{(1-fw)} \frac{g_{fM}\nu_f}{F(1-W)} + \frac{(1-f)}{(1-fw)} \frac{g_{mM}\nu_m}{(1-F)} \right] \\
 & + (1-\omega) \frac{1}{F(1-W)}
 \end{aligned} \tag{3.3}$$

The gradient of selection acting on ω , the larva's probability of developing into a worker, is the partial derivative of V_M with respect to ω . This value depends on the colony and population investment pattern (f , w). The selection pressure on ω for all f and w values in the range between the two extremes of complete worker and complete queen control is given in Figure 3.4. The selection gradient is always strongly negative, indicating that a single mutant larva always benefits to develop into a gyne, and this for all possible values of f and w .

The question arises as to what will be the equilibrium resource allocation in the colony when all larvae are in control of their developmental fate. To investigate this, we need to determine the evolutionary stable proportion of larvae (w) developing into workers and the adaptive response of colony members (workers and queens) in terms of the proportion of females f they would produce. To find the equilibrium, we have to simultaneously maximise the inclusive fitness of the larvae and the party controlling f . For larvae, we replace ω in Equation 3.3 by w (because at equilibrium all larvae adopt the same optimal strategy) and substitute the corresponding relatedness values. The resulting equation is derived with respect to w . For the party controlling f

we substitute the corresponding relatedness values in Equation 3.1 and derive with respect to f . The two derivatives are then set equal to zero and solved for f and w at $f = F = f^*$ and $w = W = w^*$.

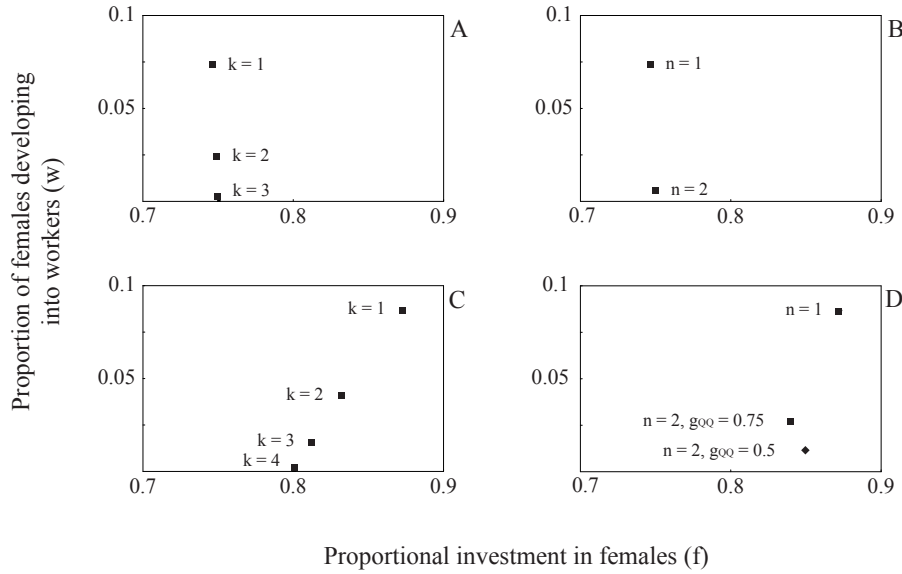


Figure 3.5: Evolutionary equilibria of the proportion of female larvae developing into workers (w) and proportional investment in females (f) a) in function of queen mating frequency (k) with queen control of proportional investment in females (f), b) in function of the number of nestmate queens (n) with queen control of proportional investment in females (f), c) in function of queen mating frequency (k) with worker control of proportional investment in females (f), and d) in function of the number of nestmate queens (n) with worker control of proportional investment in females (f). Graphs a) and c) assume monogyny and equal paternity among males, graphs b) and d) assume that queens are full sisters ($g_{QQ} = 0.75$, squares) or half-sisters ($g_{QQ} = 0.5$, diamonds), single mating, and equal contribution of queens to the production of workers, males, and gynes.

At equilibrium, the investment in workers is very low and the sex allocation strongly female biased. Under monandry, monogyny, and queen control of proportional investment in females the equilibrium is at $f^* = 0.74$ and $w^* = 0.07$, corresponding to an overall investment of 5% in workers, 69% in gynes, and 26% in males. If proportional investment in females is controlled

by workers, the equilibrium is at $f^* = 0.87$ and $w^* = 0.09$, that is 8% of the resources invested in workers, 80% in gynes, and 12% in males. The investment in workers will be even lower when queens are mated to several males (Figs. 3.5a and 3.5c) or if colonies contain several queens (Figs. 3.5b and 3.5d). The decrease associated with the higher levels of polygyny is more pronounced the less queens are related to each other.

Note that, as in previous analyses, the numerical values given here have no significance per se because they directly depend on the function $b(f, w)$ that was chosen. However, the qualitative results of the analyses are not affected by the particular function, as long as it is one with diminishing returns, and the important point is that larvae will always be in strong conflict with queens and workers over their developmental fate. Moreover, this conflict will be stronger and the proportion of larvae developing into workers lower, the higher the levels of polyandry and polygyny.

Discussion

Our model shows that potential conflict over investment in workers can arise in colonies of eusocial Hymenoptera. Although queens' and workers' inclusive fitness are maximal when the investment in workers is at the value that maximises colony productivity of sexuals (Bourke and Chan, 1999, this study), this optimum will be reached only when a single party controls all decisions about resource allocation. However, our model shows that single-party control of resource allocation is not evolutionary stable because sex ratio conflict results in strong selection on the other party manipulate investment. Hence, the queen will benefit from forcing workers to produce a less female-biased sex allocation by altering the primary sex ratio and limiting the number of eggs laid. Limitation of diploid eggs will, in turn, induce workers to direct a higher proportion of the female larvae into the gyne developmental pathway so as to achieve a more female-biased sex allocation. This tug-of-war between queens and workers results in an allocation of resources to workers that is lower than the optimal value for queens and workers, and a sex allocation intermediate between the queen and worker optima.

Resolving controversies among earlier work

Our findings shed light on the causes of discrepancies between earlier theoretical studies on the existence of potential queen-worker conflict over colony growth. Two of these studies concluded that a conflict occurs (Pamilo, 1991; Bourke and Ratnieks, 1999), whereas another reached the conclusion that there is no conflict (Bourke and Chan, 1999). Pamilo (1991) found that the queen favours a higher investment in colony growth than workers because queens value the survival of the colony more than workers (survival being higher with greater investment in workers). Workers, on the other hand, would benefit more than queens from investing in gyne production because they are more closely related to female sexuals than are queens. Pamilo's explanation is surprising because his model did not include competition among colonies. Moreover, both queens and workers should benefit most from queen-derived sexual offspring compared to less related second-generation offspring (Bourke and Chan, 1999). Similarly, Bourke and Ratnieks (1999) suggested that queen and workers should be in conflict over caste determination of individual female larvae. In their analysis the authors assumed that per-worker productivity declines with increased colony size and they graphically determined at which colony size each party switches preference from raising larvae as workers to raising them as gynes. Workers were found to favour a switch to gyne production at a smaller colony size than the queen. This is because workers are more closely related to the larvae and thus the fitness gained from producing sexual sisters outweighs the benefits of increased overall production.

A detailed analysis of these two studies provides an explanation of why they predict a queen-worker conflict. To derive the ESS, Pamilo (1991, Eq. 13) compared for each party the inclusive fitness gained from investing into workers versus gynes. This is exactly the trade-off that occurs when diploid eggs are limited, in which case workers have to decrease the investment in workers to bias sex allocation towards more females. The same is true for the analysis of Bourke and Ratnieks (1999), because they considered the specific option to direct a female larva into the gyne versus worker developmental pathway. Hence, the different queen and worker optima stem from the decision ultimately affecting colony sex allocation. By contrast, the model by Bourke and Chan (1999) assumes a trade-off between worker production versus colony productivity of sexuals (males and females) without assuming any effect of this trade-off on sex allocation. Our model shows that this is what occurs when a single party has full control over colony sex allocation and this is why they

found that queens and workers agree on the amount of resources to be directed in worker production.

Larval self-determination

Our model shows that female larvae and adult colony members are generally in strong conflict over caste determination, confirming and generalizing earlier analyses (Nonacs and Tobin, 1992; Bourke and Ratnieks, 1999). With monandry and monogyny, there is a wide range of conditions where female larvae benefit from becoming gynes, whereas queens and workers would prefer them to develop into workers. For more complex kin structure (polygyny and polyandry), the conflict is even stronger and the investment into workers lower, the predicted value being zero under a wide range of conditions. However, the prediction of no larvae developing into workers has to be considered with caution because it is dependent of the specific relationship between per worker productivity and colony size that was chosen. Moreover, our model does not include seasonal variation in the probability for gynes to successfully disperse and initiate new colonies. This may be an important factor with, for example, female larvae being more likely to develop into workers when the expected chances of successful dispersal are low.

The demonstration of a strong difference in interest between female larvae and adult individuals raises the question of who is in control of caste determination. In many species caste determination is influenced by the amount and type of food provided to larvae (Wheeler, 1986), suggesting that workers, which are usually the individuals that feed the larvae, should have a strong influence on caste determination. However, there are several means by which female larvae may gain some control over their developmental fate. First, in some species, larvae probably can influence the amount of resource they obtain and hence increase their probability of developing into a gyne (Bourke and Ratnieks, 1999). For example, in pocket-making bumblebees larvae feed autonomously in communal mass-provisioned cells (Michener, 1974; Bourke and Ratnieks, 1999). Strong competition among larvae indeed seems to occur whereby larvae occupying a central position in the communal cell monopolise food and develop more frequently into gynes than larvae in the periphery (Michener, 1974). Furthermore, workers of the pocket-making bumblebees are more variable in size than workers of pollen-storer species which rear larvae in individual cells continuously provisioned by workers (Michener, 1974). The greater variance could stem from larvae's attempts to acquire more resources in order to trigger gyne development.

An alternative route to gyne development is to decrease the caste threshold [the size at which gyne development is triggered (Wheeler, 1986)], thus allowing development into a gyne with less resources than normally required. A reduction in gyne size and caste threshold has apparently evolved in parasitic ants (Nonacs and Tobin, 1992; Aron *et al.*, 1999). In these species female larvae are under strong selection to develop into gynes because the worker tasks are performed by the host workers and worker development therefore offers only a relatively small fitness gain for parasite larvae. Reduction of queen size has also been documented in several non-parasitic Hymenoptera (Michener, 1974; Brian, 1983). Queens of reduced size (microgynes) occur for example in the bee *Trigona julianii* (Michener, 1974). Interestingly, in this species queens are usually produced in special cells but microgynes develop from workers cells (Michener, 1974). This suggests that reduced size is the only way for a female to develop into a gyne when in a worker cell. Microgynes have also been found in several ants. However, it is yet unclear whether the smaller size of these microgynes has evolved as part of a selfish strategy to increase the probability of developing into a gyne or is the result of alternative reproductive strategies (Bourke and Franks, 1995).

Finally, when a larva cannot modify the rate of food intake nor reduce size and caste threshold, another option is to increase its developmental time. However, it is unclear whether workers may counteract such selfish larval strategies, for example by reducing the amount of food provided to larvae or being aggressive towards them. Workers of some *Myrmica* species have for instance been reported to provide less food to overwintering than to spring larvae (Brian, 1983). Aggression towards larvae developing into gynes has been reported in several ant species (Fletcher, 1986; Vargo and Passera, 1991). The differential treatment of gyne-inclined larvae by workers may represent means to induce larvae to develop into workers.

Identity of the controlling parties

Our model makes predictions which may allow to test which party controls reproductive decisions in the colony. It predicts that the relative investment in workers should (i) decrease with increased levels of polygyny and polyandry if larvae can influence their developmental fate, (ii) not be affected by the levels of polygyny and polyandry if either workers or queens are in full control of resource allocation, and (iii) increase with increased levels of polygyny and polyandry if workers and queens share control over resource allocation. These

predictions can be tested by comparing the size of colonies varying in their kin structure because differences in the relative investment in workers should translate in size differences between mature colonies. Evidence for a lack of a relationship between colony size and kin structure comes from two ant species, *Formica truncorum* (Sundström and Ratnieks, 1998) and *F. exsecta* (Sundström *et al.*, 1996). In both species queens can mate singly or multiply, but colony size is apparently not associated with queen mating frequency. Interestingly, two lines of evidence suggest that workers may indeed have full control over resource allocation in these two species. First, the population wide sex investment ratio is very close to the workers' optimum (Sundström, 1994; Sundström *et al.*, 1996). Second, workers seem also to have control of reproductive decisions at the colony level because they produce the sex to which they are more related compared to the population average, as predicted by split sex ratio theory (Boomsma and Grafen, 1990, 1991). That is, colonies produce mostly females when the queen is singly mated and males when the queen is multiply mated (Sundström, 1994; Sundström *et al.*, 1996). Workers seem to attain their preferred sex allocation by selectively eliminating males in colonies headed by singly mated queens (Sundström *et al.*, 1996).

In the recent literature, queen control over sex allocation is usually indirectly inferred from an even sex allocation (e.g. Helms, 1999). However, no attempts are made to empirically investigate proximate the mechanisms of queen control that would present an alternative to the fallen paradigm of pheromonal queen control. The lack of empirical tests is surprising given that several authors have proposed the idea that queens may prevent workers from biasing sex allocation towards females by limiting the number of diploid eggs laid (Bulmer and Taylor, 1981; Ratnieks and Reeve, 1992, this study). Furthermore, the ability of queens to equilibrate the sex investment ratio by limiting diploid eggs is predicted to be lower in species with well marked gyne-worker dimorphism (Bulmer and Taylor, 1981), because in species with costly gynes even a low number of eggs raised as gynes represents a high investment in female sexuals. Thus, a higher investment in gynes does not come at the cost of a significant decrease in colony productivity due to lower worker production.

The predicted association between gyne-worker dimorphism and queen's ability to manipulate colony sex allocation is intriguing because it may provide an explanation for the observed positive correlation between sexual dimorphism and female bias across ant species. Boomsma (1989) first noted the existence of this association and suggested that it might be due to a methodological artefact with female bias in sex investment ratio tending to be

increasingly overestimated as sexual dimorphism increases (see also Boomsma *et al.*, 1995). However, the association between sexual dimorphism and female bias is also predicted if queens limit the number of female eggs because sexual dimorphism tends to be positively correlated with gyne-worker dimorphism in ants (L. Keller, unpublished). Hence, queens should have less control over sex allocation and it should be more female biased in species with greater sexual dimorphism. Experiments are needed to determine whether queen-worker conflict over sex allocation indeed leads to limitation of female eggs and whether the occurrence of egg limitation is negatively correlated with gyne-worker and gyne-male dimorphism. If such a negative correlation exists, one would expect a greater ability of workers to control resource allocation in species with a greater degree of sexual and gyne-worker dimorphism. This tendency towards complete worker control should translate into a greater investment in workers and one would thus predict a positive relationship between gyne-worker dimorphism and colony size, as indeed is the case across species of social Hymenoptera (Bourke, 1999). However, there are also other reasons to expect such an association (Bourke, 1999).

A situation similar to female egg limitation occurs when queens and males are successful in concealing the sex of the brood in order to resist the workers' manipulation of colony sex allocation (Nonacs and Carlin, 1990; Nonacs, 1993). If the deception is successful up to a developmental stage at which the fitness gain of biasing the sex allocation does not compensate for the loss of the energy already invested in rearing the males, the workers are forced to raise offspring in the sex ratio produced by the queen (Nonacs and Carlin, 1990; Chapuisat *et al.*, 1997). Under such conditions, the only reproductive decision possibly remaining under the control of the workers is the proportion of females developing into gynes versus workers, given that female caste can be influenced beyond the point where brood sex becomes apparent. There is limited evidence that sexual deception may occur in ants (Nonacs and Carlin, 1990; Chapuisat *et al.*, 1997), but well-designed experiments still need to be conducted to unambiguously demonstrate its occurrence.

Conclusion

This study explicitly delineates the conditions under which there is a conflict between queens, workers and larvae over resource allocation to the production of workers, gynes, and males. It makes predictions about how variation in queen number and queen mating frequency should affect worker production

depending on which party controls resource allocation. Our model also integrates key life-history parameters in sex allocation theory which will hopefully stimulate more empirical research on the strategies pursued by queens, workers, and female brood to maximise their inclusive fitness. These studies would help to broaden our understanding of the factors shaping the life-history of hymenopteran colonies and may provide explanations for many examples of observed sex allocation patterns that cannot be accounted for by the models of sex ratio theory available so far (Bourke and Franks, 1995; Crozier and Pamilo, 1996; Chapuisat and Keller, 1999).

Summary

The best known of the conflicts occurring in eusocial Hymenoptera is queen-worker conflict over sex ratio. So far, sex ratio theory has mostly focussed on optimal investment in the production of male versus female sexuals, neglecting the investment in workers. Increased investment in workers decreases immediate sexual productivity but increases expected future colony productivity. Thus, an important question is to determine the queen's and workers' optimal investment in each of the three castes (workers, female and male sexuals), taking into account a possible trade-off between production of female sexuals and workers (both castes developing from diploid female eggs). Here, we construct a simple and general kin selection model that allows to calculate the evolutionary stable investments in the three castes, while varying the identity of the party controlling resource allocation (relative investment in workers, female sexuals, and male sexuals). Our model shows that queens and workers favor the investment in workers that maximises lifetime colony productivity of sexuals, whatever the colony kin structure. However, worker production is predicted to be at this optimum only if one of the two parties has complete control over resource allocation, a situation which is evolutionary unstable because it strongly selects the other party to manipulate sex allocation in its favor. Queens are selected to force workers to raise all the males limiting the number of eggs they lay, whereas workers should respond to egg limitation by raising a greater proportion of the female eggs into sexuals as a means to attain a more female-biased sex allocation. This tug-of-war between queens and workers leads to a stable equilibrium where sex allocation is between the queen and worker optima, and the investment in workers below both parties' optimum. Our model further shows that, under most conditions, female larvae are in strong conflict with queens and workers over their developmental fate

because they value more their own reproduction than that of siblings. With the help of our model we also investigate how variation in queen number and number of matings per queen affect the level of conflict between queens workers and larvae, and ultimately the allocation of resource in the three castes. Finally, we make predictions that allow to test which party is in control of sex allocation and caste determination.

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Chapter 4

Sex ratio and *Wolbachia* infection in the ant *Formica exsecta*

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Introduction

Social Hymenoptera have been key organisms for the study of sex ratio theory and kin selection (Bourke and Franks, 1995; Crozier and Pamilo, 1996; Bourke, 1997; Queller and Strassmann, 1998; Keller and Chapuisat, 1999; Keller and Reeve, 1999). The haplodiploid sex determination system (with males arising from unfertilised eggs and females from fertilised eggs) results in asymmetries of genetic relatedness among colony members and a parent-offspring conflict over relative allocation of resources to the two sexes (Trivers and Hare, 1976; Nonacs, 1986; Bourke and Franks, 1995; Crozier and Pamilo, 1996). Because queens are equally related to both sexes of reproductives reared in their colony, their fitness is maximised by an even sex-ratio investment. In contrast, workers are more related to their sisters than to their brothers, and their inclusive fitness is maximised by an increased investment in female brood.

Worker control of sex allocation not only leads to a female biased population sex ratio, it can also cause variation in sex allocation between colonies with different kin-structure. Split sex ratio theory (Boomsma and Grafen, 1990) predicts that workers in colonies with a relatedness asymmetry (RA, here defined as the higher relatedness of workers to females than males in their colony) above the population average should mainly specialise in the production of females. In contrast, workers in colonies with a RA below the average should favour the production of mostly males. Several studies have confirmed this prediction (Queller and Strassmann, 1998). For example, in single-queen colonies of *Formica truncorum* and *F. exsecta*, nests headed by singly mated queens produce mostly females, whereas nests headed by multiply mated queens specialise on male production (Sundström, 1994; Sundström *et al.*, 1996). However, in both species a large part of the variance in sex allocation is not explained by variation in relatedness asymmetry. Furthermore, in a population of *F. exsecta* with multiple queens per nest and in several other species, split sex ratios occurs but colony sex ratios are not correlated with relatedness asymmetry (Keller and Chapuisat, 1999; Helms, 1999; Brown and Keller, 2000). This suggests that other factors also influence colony sex allocation. One of the potential factors accounting for variations in colony sex ratio is the amount and type of resources available, possibly because it affects the relative cost of production of males and females (Rosenheim *et al.*, 1996). In some species, variations in resource supply apparently affect sex ratio specialisation (e.g. Deslippe and Savolainen, 1995).

Recently, it has also been suggested that maternally inherited bacteria may

have an influence on sex allocation in social Hymenoptera (Crozier and Pamilo, 1993; Bourke and Franks, 1995; Crozier and Pamilo, 1996; Wenseleers and Billen, 2000). *Wolbachia*, a group of intracellular bacteria related to Rickettsiae, stand as one of the most likely candidate organism that may affect sex ratios. These bacteria are known to infect Hymenoptera and other arthropods, particularly their reproductive tissues (Rousset *et al.*, 1992a; Werren, 1997). They are transmitted through the egg cytoplasm and alter reproduction in their host in various ways. In particular, they are associated with induction of parthenogenesis, cytoplasmic incompatibility, feminisation of genetic males and male killing. Because *Wolbachia* are transmitted through females but not males, they would benefit by manipulating their host colony to produce only female sexuals and no males because males are a reproductive dead end for the bacteria. If *Wolbachia* can bias colony sex ratios but not all colonies are infected, split sex ratio could arise. Colonies bearing *Wolbachia* would produce only or predominantly females, whereas uninfected colonies would specialise on males to reduce the sex ratio bias at the population level.

The aim of this study is to determine whether *Wolbachia* infection is associated with variation in colony sex ratios in the ant *Formica exsecta*. This species has been extensively studied with regard to sex allocation and a preliminary study indicated that it harbours *Wolbachia*. Split sex ratios have been reported from two different populations that differ in their social organisation.

In a Finnish population where colonies contain a single queen (monogyny), the population sex ratio investment was female biased; most colonies headed by a singly-mated queen specialised in female production, whereas colonies headed by a multiply mated queen specialised in the production of males (Sundström *et al.*, 1996). These results are consistent with the idea that colony sex ratios are under the control of workers. By contrast, it seems unlikely that *Wolbachia* could be responsible for the association between queen mating frequency and colony sex ratios because there is no obvious reason why singly-mated queens should be more likely to be infected by *Wolbachia*.

The other study population, which is located in Switzerland, contains colonies which typically contain numerous queens (polygyny). In contrast to the Finnish population, no association between relatedness asymmetry and sex allocation was detected in the Swiss population. In that population sex ratio was highly male biased and only a minority of nests (11%) produced females (Brown and Keller, 2000, Brown and Keller submitted).

We used a PCR assay to determine the prevalence of *Wolbachia* in workers from colonies specialising in male and female production in both the Swiss and Finnish populations. We used the same colonies that had been used in previous sex ratio studies to determine whether previous conclusions on factors affecting sex ratio might have been confounded by the presence of *Wolbachia*.

Materials and Methods

Colony sampling

Our study included individuals from 71 *F. exsecta* colonies. All these colonies had been part of earlier genetic and sex ratio studies (Sundström *et al.*, 1996; Chapuisat *et al.*, 1997; Brown and Keller, 2000).

We analysed 40 nests in a Swiss polygynous population. Half of the colonies were male producing and the other half were female producing following the criteria of Brown and Keller (2000). All samples analysed were adult workers. We analysed 31 nests from a Finnish monogynous population. Colony sex ratios were obtained from Sundström *et al.* (1996). We classified colonies as male producing if they produced more than 75% males and as female producing if they produced more than 63% queens. For our study, we chose 17 female-producing and 14 male-producing nests. The age of the workers analysed varied between colonies. DNA had been extracted from adult workers in 14 of these colonies and worker pupae for the other 17 colonies. There was no significant association between the type of material analysed and colony sex ratio (Fisher's exact test, $P = 0.07$).

Wolbachia detection

We tested for the presence of *Wolbachia* in five workers from each of the selected colonies. *Wolbachia*-infection was detected by PCR-amplifying a fragment of the cell cycle gene *FtsZ* using *Wolbachia* specific primers. Primer sequences and PCR conditions are given in Wenseleers *et al.* (1998). A previous analysis (unpublished) showed that only the strain A was present in the Swiss population of *F. exsecta*. Thus, PCRs for this study were performed with primers specific for the strain A. PCR products were run on 2% agarose gels and visualised by ethidium bromide staining. Each sample was analysed twice and individuals were considered infected when positive for at least one of the PCRs.

We took several precautions to avoid artefacts in the *Wolbachia* screening. For each PCR, we used a positive as well as a negative control. The positive control was an individual known to carry *Wolbachia*. In the negative control, DNA was replaced by de-ionised water. Further, to avoid any bias of our results due to PCR conditions and/or loading effect, we alternated female- and male-producing nests in PCRs and migrations on agarose gel. Thus, in each PCR and on each gel, half of the individuals were from a female producing and the other half from a male producing nest. Finally, we could exclude false negatives due to problems during DNA extraction because all samples had previously been part of microsatellite analysis and were known to contain DNA.

Statistical analysis

Because our two samples differed with respect to locality, social organisation, and the type of material used, we performed separate statistical analysis on the data from Switzerland and Finland. We analysed the data using a generalised linear model (GLM) with a binomially distributed error and a logistic link function. Presence or absence of infection in individual workers was the dependent variable. For analysing the Swiss data, we entered the colony sex ratio (male-producing or female-producing) and colony nested within sex ratio as independent variables. For the Finnish data, we entered colony sex ratio, the material used (adult workers or pupae), the interaction of these two variables, as well as colony nested within sex ratio and material. The significance of factors was tested with Chi-squared tests. All analyses were performed with S-Plus 2000 (MathSoft Inc.).

Results

Our PCR method for detecting *Wolbachia* was reasonably reliable. In the Finnish population, 95 individuals (61%) were scored as infected for the two PCRs, 11 (7%) as infected once and 49 (32%) as never infected. Similar values were found in the Swiss population, with 96 (47%), 25 (13%) and 79 (40%) individuals being scored as being infected twice, once and never, respectively. Overall, repeated PCR amplifications gave the same result for 120 (87%) of the individuals analysed. The repeatability r of the PCR results, measured as the proportion of between-individual variance among the total variance of PCR results (Falconer and Mackay, 1996) was $r = 0.86$ and $r = 0.77$ for the

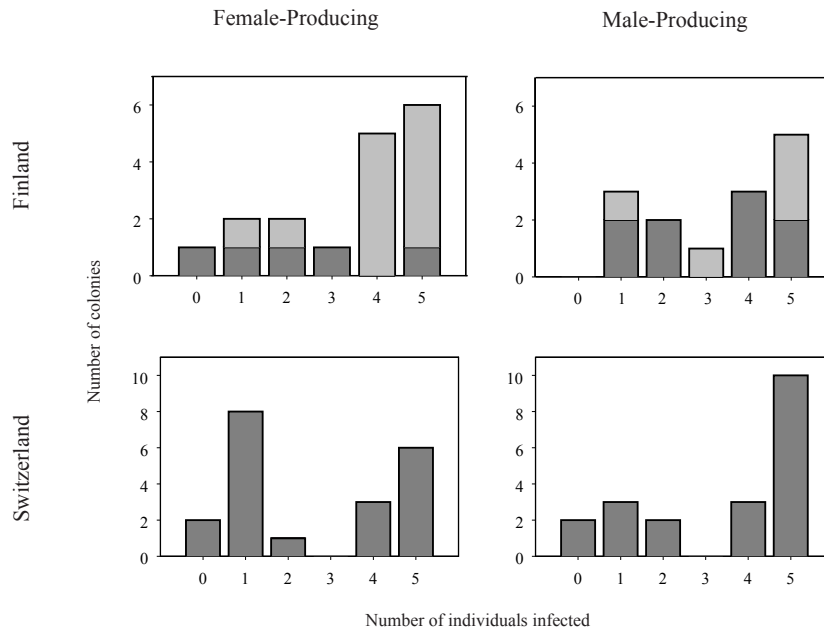


Figure 4.1: Number of infected workers in male- and female-producing colonies in the Swiss and the Finnish population. The x-axis gives the number infected workers per colony (out of the five analysed). The y-axis gives the number of colonies with a given number of infected workers. For the Finnish population, data on worker pupae are given in light grey, those on adult workers in black. In the Swiss population all individuals analysed were adult workers.

Swiss and the Finnish populations, respectively.

Most of the colonies contained some infected workers (Figure 4.1). In only four Swiss polygynous nests (10.0%) and one Finnish monogynous nest (3.2%), no infected worker was detected. In both populations there was no significant association between sex ratio and whether colonies contained infected workers (Switzerland, Fisher's exact test, $P > 0.9$; Finland, Fisher exact test, $P > 0.9$). The proportion of infected colonies did also not differ significantly between the two populations (Fisher exact test, $P = 0.38$). The overall percentage of colonies without infected workers was 7.0%. The percentage of individuals infected was similar in the two populations. Sixty and 69% of the workers were scored as infected in the Swiss and Finnish populations, respectively.

Results from the GLM analyses are presented in Table 4.1. The analyses revealed that the factor that had the greatest influence on the proportion of

Table 4.1: Results from the GLM analyses for the Finnish and the Swiss population. The dependent variable in both analyses was the infection status of workers (infected/uninfected). The table shows the independent variables entered in the analysis (Factor), the number of their degrees of freedom (df), and their significance calculated by a Chi-squared test. Further, the percentage of the total variance explained the significant factors is given (Explained V).

Factor	d.f.	<i>P</i>	Explained V
Finland			
Sex ratio	1	> 0.5	—
Material	1	0.0014	5.2%
Sex ratio x Material	1	0.2	—
Colony in (Sex ratio x Material)	27	< 0.0001	39.3%
Switzerland			
Sex ratio	1	0.0088	2.6%
Colony in Sex ratio	27	< 0.0001	58.1%

infected workers was the colony from which workers were sampled. The effect of colony was significant in both populations and explained as much as 58 and 39% of the variance in the Swiss and Finnish populations, respectively.

Overall, there was no clear association between sex ratio and the proportion of infected workers (Table 4.1). In the Swiss population, sex ratio was significantly associated with the proportion of infected workers but sex ratio explained only 2.6% of the total variance in the proportion of infected workers. Interestingly, the association was opposite to theoretical expectations if *Wolbachia* manipulated their host to increase their transmission rate. Female-producing colonies tended to have a lower number of infected workers ($2.6 \pm 2.0/1.5$; mean \pm SD / median) than male-producing colonies ($3.5 \pm 1.9/4.5$). In the Finnish population, there was no significant association between these two variables and the number of infected workers was quite similar in female- ($3.5 \pm 1.7/4.0$) and male- ($3.4 \pm 1.6/4.0$) producing colonies (Fig. 4.2).

In the Finnish population, there was a significant association between the proportion of infected workers and the type of material analysed (Table 4.1). The proportion of infected individuals was higher for pupae than workers, both in female- and male-producing colonies (Fig. 4.2). Overall, the type of material explained 5.2% of the total variance in the proportion of infected workers in Finland.

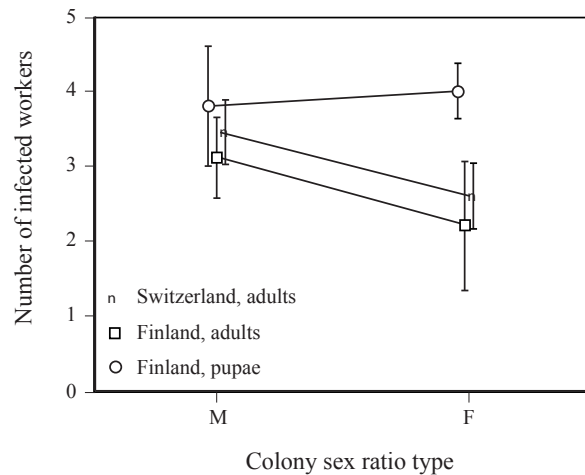


Figure 4.2: Mean \pm SE number of infected workers (out of the five analysed) in male- and female-producing colonies (M, F). Data are given separately for adult workers in the Swiss population (black circles), adult workers (white circles) and worker pupae (squares) in the Finnish population.

Discussion

Our data indicate that, in both the Swiss polygynous and Finnish monogynous populations, almost all colonies are infected with *Wolbachia*. Overall, only 7% of the colonies contained no infected workers. In the Finnish monogynous population, although only five individuals were analysed per colony, only one of the 31 colonies had no infected workers, indicating that the large majority or even all the reproductive queens are infected. In the Swiss population it is more difficult to determine the proportion of infected queens because colonies can contain large number of queens and it is impossible to accurately assign maternity of workers in such colonies. However, the finding that the average

proportion of infected workers is similar in both populations is suggestive that the proportion of infected reproductive queens might be similar in both populations, with perhaps all queens being infected.

The very high proportion of infected queens suggests that *Wolbachia* have efficient means to spread through the population. One very efficient mechanism favouring the spread of *Wolbachia* is cytoplasmic incompatibility, which occurs when an uninfected female mates with an infected male (Breeuwer and Werren, 1990; Hoffmann and Turelli, 1997). Such matings generally produce few or no female progeny as the result of abortive karyogamy (Lassy and Karr, 1996). Because the other possible types of matings yield normal progeny numbers, the outcome of cytoplasmic incompatibility is lower fitness of uninfected females compared to infected females. This reproductive advantage of infected females results in the spread of *Wolbachia* through a population (Caspari and Watson, 1959; Turelli, 1994). Interestingly, the spread of *Wolbachia* through cytoplasmic incompatibility should be even quicker in social Hymenoptera than in solitary species because uninfected females mated with infected males should produce few or no female workers (Vavre *et al.*, 2000), and hence are unlikely to be successful in founding a new colony. More studies are needed to test whether the high prevalence of *Wolbachia* in *F. exsecta* is indeed due to the effect of cytoplasmic incompatibility.

In the Swiss population the prevalence of *Wolbachia* was slightly higher in male than female producing colonies. By contrast, there was no significant association between these two factors in the Finnish population. It is important to note that the association between colony type and prevalence of *Wolbachia* in the Swiss population was weak (sex ratio explained only 2.6% of the variance). Moreover, the association was opposite to what one would predict if *Wolbachia* altered colony sex ratios so as to increase their transmission rate. Thus, it seems likely that the weak but significant association between colony type and prevalence of *Wolbachia* is not due to an active manipulation of colony sex ratio by the parasite but rather results from the effect of a confounding ecological variable that affects both sex allocation and *Wolbachia* infection.

That ecological variables or intrinsic factors can affect the proportion of infected individuals is suggested by the higher proportion of infected worker pupae than worker adults in Finland. The higher prevalence of *Wolbachia* in pupae suggests that some workers lose infection with age or that the bacterial density decreases to levels undetectable with standard PCR methods, a

result that may partially be explained by differences in the amount of fresh tissue between adults and pupae. A lower proportion of infected adult workers compared to worker pupae has also been found in another ant, *Formica truncorum* (Wenseleers *et al.*, 2002). The reasons underlying decreased proportion of infected workers with age are unknown. One possible explanation is that endoparasites are not selected to proliferate in workers that will never produce any female offspring (selection would even be negative if infection decreases worker efficiency, thus overall colony productivity). Alternatively, the proportion of infected workers may decrease as a result of the possible exposure of adult workers to higher temperature. Increased temperature has been shown to decrease the density of *Wolbachia* in *Drosophila bifasciata* (Hurst *et al.*, 2000). Thus, adult workers exposed to higher temperature while foraging may experience a decrease in the density of *Wolbachia*. It is also possible that differences in temperature (or other ecological variables) influence resource availability and thereby colony sex ratios, which might explain the weak association between these two variables in the Swiss population. Alternatively, female-producing colonies might be comprised of older workers, which would also account for an association between the proportion of infected workers and colony sex ratios.

The finding of no clear association between prevalence of *Wolbachia* and colony sex ratios together with the fact that all or almost all queens are infected indicate that sex ratio specialisation in *F. exsecta* colonies cannot simply be accounted for by the presence or absence of *Wolbachia*. In particular, the finding that colonies with more infected workers tend to produce males in the Swiss populations indicates that *Wolbachia* do not manipulate their host into producing mostly or only females. Moreover, *Wolbachia* was as prevalent in the Swiss population, which has a male-biased population sex ratio, as in the Finnish population which shows a female-biased sex ratio.

There are several possible explanations for the apparent lack of sex ratio manipulation by *Wolbachia* in *F. exsecta*. One is proximate constraints arising from the mechanism of sex determination in ants (Wenseleers and Billen, 2000). In most ants, sex is probably determined by heterozygosity at one or several sex-determination loci with heterozygous individuals developing into females and homozygous and hemizygous individuals developing into males (Cook and Crozier, 1995). Because *Wolbachia*-induced parthenogenesis generally relies on a cytogenetic mechanism of gamete duplication, yielding completely homozygous diploid offspring (Stouthamer and Kazmer, 1994) this would lead to the production of diploid males which are generally sterile. An

alternative mechanism by which *Wolbachia* might alter the sex ratio is manipulation of the proportion of viable fertilised eggs. This could be achieved if *Wolbachia* could decrease viability of haploid eggs or influence the mechanism of egg fertilisation. In *Nasonia vitripennis* a maternal-sex ratio distorter apparently induces a higher than normal frequency of egg fertilisation (Skinner, 1982). However, such an effect has not yet been reported for *Wolbachia* (in ants a possible mechanism could be by acting on the muscles regulating transfer of sperm from the queen spermatheca to the eggs). Additional studies in other ants would be helpful to ascertain whether *Wolbachia* generally has no effect on ant sex ratios. Studies of populations where *Wolbachia* has not yet spread to fixation would be particularly interesting as *Wolbachia* might have a greater effect on sex ratio variation between colonies in such non-equilibrium situation.

Summary

Sex allocation data in social Hymenoptera provide some of the best tests of kin selection, parent-offspring conflict and sex ratio theories. However, these studies critically depend on controlling for confounded ecological factors and on identifying all parties that potentially manipulate colony sex ratio. It has been suggested that maternally inherited parasites may influence sex allocation in social Hymenoptera. If the parasites can influence sex allocation, infected colonies are predicted to invest more resources in females than non-infected colonies, because the parasites are transmitted through females but not males. Prime candidates for such sex-ratio manipulation are *Wolbachia* because these cytoplasmically transmitted bacteria have been shown to affect the sex ratio of host arthropods by cytoplasmic incompatibility, parthenogenesis, male-killing and feminisation. In this study, we tested whether *Wolbachia* infection is associated with colony sex ratio in two populations of the ant *Formica exsecta* that have been the subject of extensive sex ratio studies. In these populations colonies specialise in the production of one sex or the other. We found that almost all *F. exsecta* colonies in both populations are infected with *Wolbachia*. However, in neither population did we find a significant association in the predicted direction between the prevalence of *Wolbachia* and colony sex ratio. In particular, colonies with higher proportion of infected workers did not produce more females. Hence, we conclude that *Wolbachia* does not seem to alter the sex ratio of its hosts as a means to increase transmission rate in these two populations of ants.

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Chapter 5

Multiple infection and recombination of *Wolbachia* in the ant *Formica exsecta*

The study described in this chapter has been conducted in collaboration with Laurent Keller. The manuscript is in preparation for submission to *Genetics*.

Introduction

Wolbachia are probably the most successful bacterial symbionts in nature, estimated to be present in 20-75 percent of arthropods species. *Wolbachia* are intracellular symbionts belonging to the alpha-proteobacterian group Rickettsiae. As cytoplasmic elements, their predominant mode of transmission is vertical. Along with mitochondria they are passed from the mother to the offspring. Paternal transmission through sperm is probably very rare (Turelli *et al.*, 1992; Turelli and Hoffmann, 1995). Despite their maternal inheritance within host populations, *Wolbachia*'s spread between host species seems to rely on horizontal transmission. Accordingly, the phylogeny of *Wolbachia* differs markedly from that of its hosts (Werren *et al.*, 1995a,b; O'Neill *et al.*, 1992; Rousset *et al.*, 1992a; Cook and Butcher, 1999).

Wolbachia can not only be found in a large number of host species, they also attain a high prevalence within host populations. The spread of infection is promoted by symbiont-induced alterations of host reproductive physiology. Four different symbiont effects, called phenotypes, have been described in Arthropods (see Werren, 1997). All of these increase infection frequency by assuring that the number of infected daughters produced by an infected female exceeds the average production of daughters per female. Three phenotypes achieve this goal by increasing the proportion of females among an infected mother's offspring, either by causing genetic sons to develop into functional daughters (feminisation phenotype), inducing parthenogenetic reproduction in the mother (parthenogenesis induction), or by provoking the selective abortion of sons (male-killing). The latter increases survival of female offspring in species with sib competition for resources. The fourth phenotype, cytoplasmic incompatibility (CI), renders matings between infected males and uninfected females partly sterile. Incompatible matings promote the spread of *Wolbachia* infection because they lower the expected productivity of uninfected females as compared to infected females, the latter reproducing normally with both infected and uninfected males.

The spread of a CI-inducing *Wolbachia* through a host population depends on intrinsic properties of the symbiont strain and/or host-symbiont interactions, such as the degree of incompatibility caused by the symbiont or its effects on host fitness (Caspari and Watson, 1959; Hoffmann and Turelli, 1997). In addition, the dispersal behaviour of the host is expected to play an important role for infection dynamics in discontinuous host populations. Hosts dispersal has a direct impact on the frequency of incompatible matings and

dispersal of female hosts also mediates the transfer of symbionts between sub-populations. Of special interest are therefore *Wolbachia* infections in host species where dispersal rates are reduced, such as the ant *Formica exsecta*. A population of these ants, situated in the Swiss Jura mountains, has been shown to harbour *Wolbachia* (Chapter 4). The symbionts most probably cause the CI phenotype, because despite being infected, most colonies produce exclusively male sexuals. Moreover, contrary to what would be expected with all other phenotypes, the sex ratio of sexual offspring produced is not correlated with *Wolbachia* prevalence within colonies (Chapter 4). A population genetic study of the host population revealed that dispersal of ant queens occurs at very low rates (Liautard and Keller, 2001). The ant population covers several forest clearings, which form distinct sub-populations. Mitochondrial genetic markers are highly differentiated between these sub-populations, most of which contain ants with an identical haplotype. In the few sub-populations where several haplotypes co-occur, these are clustered locally. This indicates that also within sub-populations dispersal occurs at a small local scale. The present study revealed high levels of multiple *Wolbachia* infection in the ant population, with all ants carrying between four and five different *Wolbachia* lineages. Hosts varied in the number of strains they carried, because one *Wolbachia* strain was exclusively associated with three host mitochondrial haplotypes while lacking in the two remaining mitochondrial lineages. This difference in infection between haplotypic lineages was independent of locality and could be detected even between ants sampled from the same population. Finally, an analysis of the *wsp* gene sequences of the *Wolbachia* strains carried by *F. exsecta* revealed that three of the five strains are most probably the result of homologous recombination.

Material and Methods

We used DNA samples that had already been employed by Liautard and Keller (2001). For their study, workers had been collected in different nests from nine locations within the Swiss population of *F. exsecta*. DNA of the ants had been extracted following a standard salt extraction protocol. With the help of three RFLP markers within the cytochrome B and ND2 genes Liautard and Keller (2001) had determined the mitochondrial haplotypes of the workers.

Table 5.1: Results of RFLP analysis. The table gives for each individual the haplotype, the locality and nest of origin. *Wolbachia* lines detected are indicated by a cross (X). Bold face indicates individuals used for sequencing of *Wolbachia*.

Haplotype	Locality	Nest	wFex1	wFex2	wFex3	wFex4	wFex5
Hap1	BA	2	-	X	X	X	X
Hap1	BA	4	-	X	X	X	X
Hap1	BA	10	-	X	X	X	X
Hap1	DU	1	-	X	X	X	X
Hap1	DU	5	-	X	X	X	X
Hap1	PJ	1	-	X	X	X	X
Hap1	PJ	6	-	X	X	X	X
Hap1	Chen	8	-	X	X	X	X
Hap1	Chen	74	-	X	X	X	X
Hap1	Chen	213	-	X	X	X	X
Hap2	BB	2	X	X	X	X	X
Hap2	BB	6	X	X	X	X	X
Hap2	BB	10	X	X	X	X	X
Hap2	PN	1	X	X	X	X	X
Hap2	PN	5	X	X	X	X	X
Hap2	PN	8	X	X	X	X	X
Hap2	ROC	3	X	X	X	X	X
Hap2	ROC	7	X	X	X	X	X
Hap2	ROC	10	X	X	X	X	X
Hap3	PJ	3	-	X	X	X	X
Hap3	PJ	4	-	X	X	X	X
Hap3	PJ	7	-	X	X	X	X
Hap3	LO	1	-	X	X	X	X
Hap3	LO	2	-	X	X	X	X
Hap3	LO	6	-	X	X	X	X
Hap4	PN	1	X	X	X	X	X
Hap4	PN	2	X	X	X	X	X
Hap6	LC	1	X	X	X	X	X
Hap6	LC	5	X	X	X	X	X
Hap6	LC	8	X	X	X	X	X
Hap6	LO	3	X	X	X	X	X
Hap6	LO	4	X	X	X	X	X
Hap6	LO	5	X	X	X	X	X
Hap6	PN	9	X	X	X	X	X

Sequencing

In order to obtain an overview of the *Wolbachia* lineages occurring in the ant population, we first sequenced the *wsp* gene of *Wolbachia* harboured in individuals with different mitochondrial haplotypes. Based on data from Liautard and Keller (2001) we chose one individual with from each of the haplotypes Hap1, Hap2, Hap3, Hap4, and Hap6 (Tab. 5.1, individuals in bold face). A sixth haplotype reported by Liautard and Keller (Hap8) occurred only in a very small number of individuals from a single sub-population and was not considered here. While choosing the samples, care was taken to use individuals from different localities. Wherever possible, individuals were chosen from sub-population in which one haplotype was fixed (Hap1 from sub-population BA, Hap2 from BB, Hap6 from LC; see Liautard and Keller (2001) for more details about the locations). Haplotypes Hap3 and Hap4 are fixed in none of the sub-populations investigated by Liautard and Keller. We therefore chose individuals from the location where the haplotype was represented in the highest proportion (Hap3 from sub-population PJ, Hap4 from PN).

In order to sequence the *wsp* gene from individual *Wolbachia* within the hosts, the gene was amplified from the extractions of ant DNA using primers *wsp* 81F and *wsp* 691R (Braig *et al.*, 1998) and following the protocol by Zhou *et al.* (1998). The PCR product was run on 1.5% agarose gels and visualised by staining with SYBR Gold (Molecular Probes). Individual bands were cut out of the gel and frozen at -20°C. DNA was recovered out of the thawed gel slices by spinning in a table-top centrifuge at maximum speed for about five minutes and cloned using a TOPO TA Cloning Kit with TOP 10 One Shot chemically competent cells (Invitrogen). *E. coli* colonies were selected if the *wsp* gene could be successfully amplified out of the plasmids using the same PCR conditions as the initial amplification. Bacteria from selected colonies were grown over-night in liquid LB medium containing ampicillin and the plasmid was recovered with the Wizard Plus SV Minipreps DNA Purification System (Promega). Sequencing reactions were performed in one or both forward and reverse directions using the ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and run on an ABI 377 automated sequencer. Forward and reverse sequences from same clones were aligned and corrected by hand in order to obtain an unambiguous sequence for each clone. We sequenced between 12 and 14 clones per host individual, i.e., a total of 65 clones.

Sequence analysis

Sequences were aligned by eye in Se-Al (A. Rambaut, evolve.zoo.ox.ac/software) while taking into account their amino acid translation. Analyses of sequence divergence were performed using PAUP 4.0b8 (D. L. Swofford). To test the statistical significance of the recombination events we identified in our data, we employed a maximum likelihood method developed by Holmes *et al.* (1999). The test determines the probability of a recombination event in a set of three sequences, a recombinant and two putative "parents". A statistical parameter is calculated based on maximum likelihood tree construction. This parameter is compared to a null distribution obtained by applying the test to sequence sets generated by simulating clonal evolution (excluding recombination) under the same maximum likelihood model as that used for the original test. We performed the test with the program LARD (Holmes *et al.*, 1999, evolve.zoo.ox.ac/software), the null distribution was generated by simulating 200 sequence sets with Seq-Gen (Rambaut and Grassly, 1997, evolve.zoo.ox.ac/software). The maximum likelihood estimations of phylogenetic parameters needed for the test and generation of the null distribution were obtained by applying a HKY85 model (Hasegawa *et al.*, 1985) including gamma-distributed rate heterogeneity between sites to the ensemble of our sequences in PAUP version 4.0b8 (D. L. Swofford).

RFLP genotyping

In order to verify whether the *Wolbachia* lineages identified by sequencing were differentially associated with host mitochondrial haplotypes, we used RFLP to detect *Wolbachia* lineages in a larger sample of ants. For each of the haplotypes Hap1, Hap2, Hap3, Hap4, and Hap6 we analysed ants from several nests from all localities where that haplotype occurred. The sampling scheme is summarised in Table 5.1.

Two enzymes, RsaI and HindIII, cut *wsp* sequences of the different *Wolbachia* lineages in a way that produced one specific band for each lineage. For RFLP analysis, the *wsp* gene was amplified with primers *wsp* 81F and *wsp* 691R. The reaction was carried out in a volume of 50 μ l, containing, 0.5 μ M of both primers, 250 μ M of each nucleotide, 2.5mM of MgCl₂, 1.25U of SuperTag Plus (HT Biotechnology), 1x buffer, and 8 μ l of DNA extract. The PCR temperature profile consisted of an initial denaturation at 92°C for three minutes, thirty cycles of 30 seconds denaturation at 92°C, 1 minute of annealing at

55°C, and 1 minute of extension at 68°C, followed by a final extension of 5 minutes at 68°C. PCR products were purified with QiaQuick spin columns (Qiagen) and re-suspended in 30µl sterile water. The whole amplified DNA was digested in 50µl containing 3.3U RsaI (Appligene), 3U HindIII (Gibco), and 1x buffer 2 (Gibco). Digestion was carried out at 37°C during two hours. Fragments were run on a high resolution gel Spreadex EL1200 (Elchrom Scientific) at 55°C and 10V/cm during 70 minutes. Gels were stained with SYBR Gold (Molecular Probes) and destained in water before photographing.

Nucl. pos.	102	104	105	107	108	109	110	111	112	115	116	117	118	119	123	126	129	132	139	143	144	146		
wFex1	A	G	C	A	A	G	A	C	A	A	A	T	A	G	C	A	A	A	A	C	T	G		
wFex2	A	A	C	G	T	A	G	T	G	T	A	C	A	G	A	A	G	G	A	C	T	G		
wFex3	A	A	C	G	T	A	G	T	G	T	A	C	A	G	A	A	G	G	A	C	T	G		
wFex5	G	G	G	C	T	G	A	T	G	G	T	T	G	A	T	T	A	T	C	G	A	T		
wFex4	G	G	G	C	T	G	A	T	G	G	T	T	G	A	T	T	A	T	C	G	A	T		
Nucl. pos.	147	152	153	156	165	180	183	189	190	198	199	201	204	208	209	210	211	213	216	219	220	224		
wFex1	C	G	T	G	T	C	G	C	G	A	C	T	C	T	G	G	T	G	T	A	G	C		
wFex2	T	G	T	A	C	C	G	T	G	A	G	T	T	T	A	C	C	A	C	A	A	A		
wFex3	T	G	T	A	C	C	G	T	G	A	G	T	T	T	A	C	C	A	C	A	A	A		
wFex5	T	C	C	A	T	T	A	T	A	G	C	A	T	C	A	G	C	A	C	G	A	T		
wFex4	T	C	C	A	T	T	A	T	A	G	C	A	T	C	A	G	C	A	C	G	A	T		
Nucl. pos.	225	226	227	228	229	230	231	233	234	236	237	238	239	240	241	242	243	244	245	246	247	248		
wFex1	A	G	A	T	G	T	A	T	A	G	T	-	-	-	-	-	-	-	-	-	-	-		
wFex2	T	G	T	T	A	C	A	G	T	C	A	C	A	A	C	A	T	T	T	A	C	G	C	C
wFex3	T	G	T	T	A	C	A	G	T	C	A	C	A	A	C	A	T	T	A	C	G	C	C	C
wFex5	G	A	A	C	A	A	T	A	T	A	A	G	T	G	C	T	T	A	C	T	C	C	C	
wFex4	G	A	A	C	A	A	T	A	T	A	A	G	T	G	C	T	T	A	C	T	C	C	C	
Nucl. pos.	249	255	256	261	262	263	264	267	272	285	286	294	307	312	321	348	367	369	372	375	382	383		
wFex1	-	A	G	A	G	A	A	C	C	A	T	C	G	G	C	T	A	T	C	A	G	C		
wFex2	A	T	A	G	A	A	C	T	C	A	C	C	A	A	T	T	A	T	C	A	G	C		
wFex3	A	T	A	G	A	A	C	T	C	A	C	C	A	A	T	C	G	C	T	T	A	A		
wFex5	A	T	G	G	G	G	C	C	A	G	C	T	A	A	T	T	A	T	C	A	G	C		
wFex4	A	T	G	G	G	G	C	C	A	G	C	T	A	A	T	C	G	C	T	T	A	A		
Nucl. pos.	385	386	387	391	393	395	397	405	406	407	416	424	425	434	441	446	450	466	474	489	503	513		
wFex1	A	C	T	G	G	G	A	T	G	G	C	G	G	G	T	G	C	G	T	C	A	C		
wFex2	A	C	C	A	G	G	A	T	G	G	C	G	G	G	T	G	C	G	T	C	A	C		
wFex3	G	A	A	A	A	A	G	A	A	A	G	T	A	A	G	A	T	A	C	T	G	T		
wFex5	A	C	C	A	G	G	A	T	G	G	C	G	G	G	T	G	C	G	T	C	A	C		
wFex4	G	A	A	A	A	A	G	A	A	A	G	T	A	A	G	A	T	A	C	T	G	T		
Nucl. pos.	516	520	521	522	523	525	526	529	530	531	535	537	539	540	546	548	551	552	553	554	559	584		
wFex1	T	-	-	-	A	A	A	G	A	C	G	C	G	C	G	A	T	C	A	A	C	C		
wFex2	T	-	-	-	A	A	A	G	A	C	G	C	G	C	G	A	A	C	A	A	C	C		
wFex3	C	A	G	C	G	T	G	A	G	T	A	A	A	T	A	G	A	T	G	C	T	T		
wFex5	T	-	-	-	A	A	A	G	A	C	G	C	G	C	G	A	D	C	A	A	C	C		
wFex4	C	A	G	C	G	T	G	A	G	T	A	A	A	T	A	G	A	T	G	C	T	T		

Figure 5.1: Figure 1: Sequence variation in the wsp gene of the five Wolbachia lineages identified in *F. exsecta*. Position 1 is equivalent to position 386 in the original wsp sequence by Braig *et al.* (1998).

Results

We identified a total of five different *Wolbachia* strains. The strains have been named wFex1–wFex5 and their *wsp* sequences can be accessed on Genbank under the numbers AY101196, AY101197, AY101198, AY101199, and AY101200, respectively. Blasting the sequences in Genbank identified members of the *Wolbachia* A-group as closest matches. Furthermore, blasts revealed that the *wsp* sequence of wFex1 is identical to that of a *Wolbachia* carried by the tephritid fruit fly *Dacus destillatoria* (accession number AF295344) whereas wFex4's *wsp* gene is identical to that of a *Wolbachia* sequenced in the ant *Formica truncorum* (accession number AF326978). The closest match to wFex2 was the *wsp* sequence of *Wolbachia* strain wUni, identified in *Muscidifurax uniraptor* (accession number AF020071). However, the wFex2 and wUni differed at almost three percent of nucleotide positions. For wFex3 and wFex5, no close matches were found, the most similar sequences differed by 7–8% of nucleotides.

The similarities between the *wsp* sequences of the five wFex lineages suggest that three of them have arisen by homologous recombination. Strains wFex3 and wFex5 share large parts of identical sequences with strains wFex2 and wFex4. As shown in Figure 5.1, the left part of wFex3's *wsp* sequence is identical to that of wFex2, up to a breakpoint situated between nucleotide positions 321 and 348. To the right of this point, the sequence of wFex3 is identical to that of wFex4. The *wsp* sequence of wFex5 shows exactly the reversed pattern of similarity, with a 5'-part identical to wFex4 and a 3'-part identical to wFex2. The LARD analysis gave strong statistical support for the recombinational origin of *wsp* sequences of wFex3 and wFex5. In tests using wFex2 and wFex4 as parental taxa and either one of the recombinant strains, none of 200 simulated sequence sets produced a likelihood ratio greater than that obtained on the original data sets. This corresponds to a significance level of $P < 0.005$. Recombination might also be at the origin of wFex1 or wFex2. The 3'-parts of their *wsp* sequences differ by only two nucleotides whereas they differ by 16% of nucleotides on the 5'-end (nucleotides 1–321). A significance test for this recombination event could not be performed because of the lack of a second parental sequence.

The RFLP analyses revealed high levels of multiple *Wolbachia* infection (Tab. 1). All ants analysed harboured symbionts from strains wFex2, wFex3, wFex4, and wFex5. Lineage wFex1 was found in all hosts with mitochondrial haplotypes Hap2, Hap4, and Hap6 but lacked in all ants with haplotypes Hap1 and Hap3. The variation in infection between haplotypes was completely in-

dependent of locality. Ants with the same haplotype but sampled in different nests or sub-population harboured the same symbiont lineages, while individuals from the same locality but belonging to different haplotype groups (Hap1 and Hap3 vs. Hap2, Hap4 and Hap6) differed in infection (Tab. 5.1).

Discussion

Our study revealed high levels of multiple *Wolbachia* infection in the ant *F. exsecta*. All ants were found to harbour four or five *Wolbachia* strains. This number is very high compared to other multiply infected hosts, which have been found to harbour two or three strains. For example, two co-existing strains have been found in *Nasonia vitripennis*, *N. giraulti*, *N. longicornis* (Breeuwer *et al.*, 1992), *Drosophila simulans* (Merçot *et al.*, 1995), *Aedes albopictus* (Sinkins *et al.*, 1995), and *Trichopria sp.* (Vavre *et al.*, 1999). Three *Wolbachia* strains have so far only been reported from three host species, the beetle *Callosobruchus chinensis* (Kondo *et al.*, 2002) and the parasitoid wasps *Leptopilina heterotoma* and *Asobara tabida* (Vavre *et al.*, 1999). It is not clear what factors are at the origin of the exceptionally high level of multiple infection in *F. exsecta*. One possibility might be that multiple infection is the result of repeated invasion of different *Wolbachia* strains, combined with the generation of new strains by recombination. The strains wFex1, wFex2, and wFex4 are more likely to have invaded the ant population, because identical or very similar *Wolbachia* strains could be found in other insect species. Strains wFex3 and wFex5, on the other hand might have arisen subsequently by recombination within *F. exsecta*. The LARD analysis has identified the two strains as probable recombinants between wFex2 and wFex4. Furthermore, no close correspondence to the two putative recombinant strains could be found among the *Wolbachia* strains for which wsp sequences have been obtained so far. Although the interpretation of recombination within *F. exsecta* is consistent with our data, it is impossible to confirm at the present stage. The fact that *Wolbachia* strains sharing an identical wsp sequence can be found in very distant host species makes clear that recombinant strains might equally well have been generated elsewhere and later have invaded the ant population.

The present study stresses the importance of recombination in generating new symbiont strains. Although we cannot determine with certainty in which host recombination has taken place, we have strong evidence that at least two but most probably three of the five wFex strains are the result of homologous

recombination. Recombination might thus not be a rare and exceptional event. This brings about the question of how a recombinant strain, that initially is present in a single host individual, can spread through the host population? One possibility is that recombinant strains spread by random drift. In this case only a very small proportion of recombinant strains would ever become fixed, because most of them would get lost when initially rare. The fact that several recombinant strains have successfully spread would thus indicate that recombination must occur very frequently. A second possibility is that recombinant strains increased in frequency due to a selective advantage. One such advantage would be an increased rate of vertical transmission. The fact the recombination events described here are situated within the gene *wsp* might be important in that respect. *wsp* codes for a surface protein and might be involved in host-symbiont interactions (Werren and Bartos, 2001; Jiggins *et al.*, 2001). If carrying a new type of surface protein allows recombinant strains to better escape host control, this might represent a selective advantage for the recombinant and thus explain their spread.

Another intriguing result of this study is that the breakpoints of the recombination events identified fall into a stretch of less than thirty base pairs. This suggests that the site of recombination might not be random. Regions in the genome where the rate of recombination is very high ("recombination hot-spots") have been described in several species (Myers and Stahl, 1994). Recombination hot-spots are characterised by the presence of so-called crossover hot-spot instigator (*Chi*) sequences. *Chi* are short sequences that trigger the transformation of the exonuclease RecBCD into the recombinase RecBC(D⁻) (Myers and Stahl, 1994; Kowalczykowski, 2000). *Chi* sequences are found within regions of the genome which are rich in GT-content and facilitate the binding of RecA, an enzyme essential for recombination in *E. coli* (Lao and Forsdyke, 2000; Tracy *et al.*, 1997). Interestingly, all our recombination events fall into a conserved region containing a 19 base pair stretch consisting exclusively of G and T.

Our RFLP analyses have shown that *Wolbachia* strain wFex1 systematically lacked in hosts with mitochondrial haplotypes Hap1 and Hap3 but was in all hosts with the three other haplotypes. This result is surprising given that the *Wolbachia* harboured by *F. exsecta* are thought to cause CI. Following theoretical models of the dynamics of CI-inducing *Wolbachia* infections, host maternal lineages lacking symbiont strains should be rapidly eradicated from the population, because they suffer a selective disadvantage due to incompatible matings (Hoffmann and Turelli, 1997). There are two possible explanations

for how the haplotypic lineages Hap1 and Hap3 could nevertheless be maintained in the population. First, females lacking strain wFex1 might not suffer incompatible matings because they do not interbreed with males carrying that strain. This is unlikely because hosts carrying wFex1 (Hap2, Hap4, Hap6) and those lacking it (Hap1, Hap3) can be found in the same population and even in the same nest (Liautard and Keller, 2001). Second, the *Wolbachia* strain wFex1 might not cause incompatibility. Neutral strains that neither cause CI nor protect from it are known from *Drosophila simulans* (Hoffmann *et al.*, 1996) and *D. mauritiana* (Giordano *et al.*, 1995). How these strains have been able to invade the host population and how they are maintained remains a puzzle (Hoffmann and Turelli, 1997). It has been suggested that neutral strains might hitch-hike during invasion of another CI-causing *Wolbachia* lineage (Giordano *et al.*, 1995). Thus, wFex1 might have spread because it was initially associated with one of the other strains which caused CI. Giordano *et al.* (1995) state that once fixed, a neutral strain could maintain itself in the host population if it was beneficial to their host. If wFex1 had no positive effect on the host, this could explain why it has been lost in the haplotypic lineages Hap1 and Hap3. The scenario of wFex1 spreading in association with another CI-inducing strain and being subsequently lost in some host lineages assumes that the *Wolbachia* infection is older than the divergence of host mitochondrial haplotypes. Although we lack direct evidence, our data are in agreement with this hypothesis. The clear association of strain wFex1 with certain host mitochondrial haplotypes indicates that *Wolbachia* is almost exclusively or always vertically transmitted. This, together with the fact that the other four *Wolbachia* strains are present in hosts of all mitochondrial haplotypes, indicates that mitochondrial haplotypes have most probably diverged after *Wolbachia* infections had been fixed.

In conclusion, this study suggests that the infection dynamics of CI-causing *Wolbachia* might be more complex than hitherto appreciated. Thus, recombination in *Wolbachia* might occur relatively frequently. Due to our poor understanding of *Wolbachia*-host interactions it is currently difficult to appreciate the importance of recombination for the evolution of the symbiont as well as the co-evolution with its host. In the future, increasing knowledge about *Wolbachia*'s genome as well as the molecular basis of *Wolbachia*-host interactions will allow us to more fully apprehend the role of selection and recombination in the evolution this fascinating symbiont.

Summary

Wolbachia bacteria are intracellular symbionts of many arthropod species. Their spread through host populations is promoted by the drastic alterations that they impose on host reproductive physiology. In the present study, we analysed the association between *Wolbachia* and host mitochondrial genotypes in the ant *Formica exsecta*. A joint analysis of the two maternally inherited elements allows to make inferences on the history of infection as well as the exact mode of transmission of *Wolbachia*. Furthermore, *F. exsecta* is particular because female dispersal occurs at extremely low rates. Mitochondrial genotypes are therefore strongly differentiated between and within populations. Our study revealed exceptionally high levels of multiple infection, with all individual ants harbouring between four and five different symbiont strains. Interestingly, hosts varied in the number of strains they carried, because one *Wolbachia* strain was exclusively associated with three host mitochondrial haplotypes while lacking in the two remaining mitochondrial lineages. Furthermore, sequence analyses of the *Wolbachia* gene *wsp* revealed that three of the five *Wolbachia* strains have probably arisen by recombination.

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Chapter 6

Conclusion

The results presented in this thesis illustrate the importance of multi-level selection in the evolution of co-operation and conflict. This is particularly clear in the first part which treats the evolution of queen-worker conflict over sex allocation. Chapters 2 and 3 demonstrate that selection at the colony level can restrain selfish strategies, if these have a negative effect on overall colony performance. Costs caused by the workers' sex ratio adjustment, for example, will select against sex ratio biasing. Consequently, sex allocation under worker control deviates from the equilibrium predicted on the basis of relatedness asymmetry alone. Furthermore, the degree to which queens can counter-manipulate sex ratio biasing by workers might equally be limited by the effect of their actions on colony productivity. Selection at the colony level might thus prevent queens from attaining their optimal sex allocation in the same way as it does with workers. In extension of this argument, the existence of a compromise between workers and queens would require higher-level selection. Only then the tug-of-war between workers and queens would be limited by the impact that the parties' strategies have on group productivity.

Chapters 2 and 3 also stress the importance of including proximate mechanisms into sex ratio theory. The vast majority of models on optimal sex allocation in eusocial Hymenoptera have focused on genetic relatedness as the sole factor driving the evolution of colony investment. These models have been of great importance in identifying queen-worker conflict and generating predictions on optimal sex allocation for a wide range of mating systems and population structures. Furthermore, they have triggered a large number of empirical studies on colony sex ratios. These studies could often qualitatively confirm the predictions made, but much variance remains unexplained. At this stage, introducing proximate aspects of queen-worker conflict in sex ratio theory seems of great importance for three reasons. First, refined models including proximate mechanisms and the costs they entail can contribute to

resolve the residual variance in sex allocation that we observe between species, populations, or colonies (Chapter 2). Second, thinking about proximate mechanisms will allow us to identify the possibilities that the two parties actually have to influence colony investment. Only if we have identified these, we will be able to predict the outcome of conflict. Third, identifying the proximate mechanisms of sex ratio biasing allows us to relate genetic conflict to the basic biology of social insect species. Chapter 3 demonstrates this by linking the conflict between queens, workers, and female larvae to colony growth, a fundamental aspect of life-history.

The second part of this thesis has provided some insight in the association between *Wolbachia* symbionts and their ant host *Formica exsecta* — even if the two studies conducted so far have provoked more questions than they have answered. Chapter 4 tempted to determine the effect of *Wolbachia* on its host by testing whether the presence of the symbiont was associated with variations in colony sex allocation. Because *Wolbachia* are maternally transmitted, we expected the prevalence of *Wolbachia* infection to be higher in colonies which produced females than in those which did not. This was not the case. The question what *Wolbachia* does in *Formica exsecta* remains therefore unresolved. Assuming that the symbionts' effects on the host are restricted to those observed in other arthropod hosts, we can by exclusion identify cytoplasmic incompatibility (CI) as the most probable phenotype. All other phenotypes — male-killing, parthenogenesis induction, and feminisation — should have resulted in an association between prevalence of *Wolbachia* infection and colony sex ratio. The hypothesis of CI as the most probable phenotype is corroborated by the finding that *F. exsecta* harbours multiple symbiont strains, which so far has only be observed in host-symbiont associations expressing CI (Chapter 5). However, crossing experiments are needed to confirm the hypothesis of a CI phenotype. CI would be supported by observing infertility in matings between males carrying all *Wolbachia* strains and females that lack some of them, for example due to incomplete maternal transmission.

Crossing experiments could also elucidate the interesting finding that *Wolbachia* strain wFex1 is associated with some host mitochondrial haplotype but not others. Under the assumption that the *Wolbachia* harboured by *F. exsecta* cause CI and for reasons outlined in Chapter 5, the variations in infection between host haplotypic lineages suggest that this *Wolbachia* strain causes only weak or no CI. It could therefore have been lost in two maternal lineages without these hosts suffering significant losses due to incompatibility. Conducting crosses between females lacking wFex1 and males carrying it would

allow to verify this scenario. If wFex1 does effectively not cause CI, these crosses should be as fecund as crosses between pairs of ants carrying the same *Wolbachia* strains. Taken together, the results of the two crossing experiments could also permit to make inferences about conflict between host and *Wolbachia*. Confirming that wFex1 does not cause CI while the other strains (fixed in the host population) do, we could conclude that conflict is probably absent, because all possible matings are compatible and *Wolbachia* therefore does not interfere with host reproduction. *Wolbachia* thus would not cause any fitness loss in the host.

Finally, the study presented in Chapter 5 revealed that three of the five *Wolbachia* strains harboured by *F. exsecta* were the product of homologous recombination. The discovery that *Wolbachia* symbionts recombine is very recent (Werren and Bartos, 2001; Jiggins *et al.*, 2001) and very little is known about the importance of this phenomenon for the evolution of the symbiont and its interactions with the host. This raises three questions. First, how frequent is recombination? Detailed phylogenetic studies aiming at identifying potential recombinant strains could provide information on this point. Performing these analyses on several genes independently could answer a second question, whether recombination occurs uniformly over the whole *Wolbachia* genome or whether it is restricted to certain genes or regions of the genome. It has been hypothesised that recombination might be of particular importance in genes involved in host-symbiont interactions, such as the gene *wsp* used in Chapter 5 (Werren and Bartos, 2001; Jiggins *et al.*, 2001). New genetic variants emerging through gene exchange between *Wolbachia* lineages would perhaps be better able to escape host control and manipulation. If this was true, one would expect to find a dis-proportionally high rate of recombination in the gene *wsp*. This gene codes for a surface protein and has therefore been hypothesised to play an important role in host-symbiont interactions (Werren and Bartos, 2001; Jiggins *et al.*, 2001; von der Schulenburg *et al.*, 2000). Finally, correlating the number of recombinants identified in a certain gene with the GT-content of that genes might give some information about the control of recombination. An association between recombination rate and GT-content would hint towards a possible role of *Chi*-sequences in the control of recombination, because *Chi* appears to be situated in GT-rich islands (Lao and Forsdyke, 2000; Tracy *et al.*, 1997).

As an overall conclusion, by the variety of its topics and approaches this thesis has perhaps not given a cohesive and conclusive analysis of conflict in animal societies. Nevertheless, it may have deepened our understanding of the

functioning of biological alliances. It has done so by making contributions to both fields that it englobed, the analysis of hymenopteran sex ratio conflict and the study of *Wolbachia*-host associations. The contribution to the first field consists in introducing a more mechanistic perspective in sex ratio theory, which allows for more comprehensive analysis of the evolution of sex allocation but also approaches theory more to the biology of Hymenopteran species. The contribution to the study of *Wolbachia* consisted in the identification of a number of interesting phenomena, the detailed study of which will shed some light on the evolutionary processes that govern the dynamics of *Wolbachia* infections as well as the evolution of the symbiont and its interactions with the host.

Abstract / Résumé

Abstract

The present work investigates the interaction between members of animal associations. Two examples are studied, insect societies and the association between the ant *Formica exsecta* and its intracellular symbiont *Wolbachia*. Both associations are characterised by the co-existence of co-operation and conflict.

Social insect colonies are a prime example of co-operation. Nevertheless, queens and workers are in disagreement over a number of reproductive parameters. Conflict over sex allocation has probably found the most attention. Due to relatedness asymmetries brought about by the haplodiploid system of sex determination, queens favour an even investment in male and female sexuals whereas workers prefer a female-biased investment. Sex-ratio conflict has been extensively studied from a theoretical point of view. So far, however, most models were based on relatedness asymmetry alone. The first part of this thesis (Chapters 1 and 2) introduces the proximate mechanisms by which workers and queens manipulate sex allocation into models of optimal sex allocation. It is shown that proximate mechanisms, due to their negative effects on colony productivity, can cause sex allocation to deviate significantly from the predictions made by classical models. Furthermore, the consideration of proximate mechanisms allows to investigate how the opposing parties are selected to gain power over colony investment. Finally, the models provide a prediction on the outcome of conflict, i.e., a compromise at which queens and workers share control of investment.

The second part of this thesis investigates a *Wolbachia* infection in the ant *Formica exsecta*. *Wolbachia* are maternally inherited cytoplasmic symbionts of many arthropod species. They alter the host's reproductive physiology in order to promote their spread through the host population. Chapter 3 describes a study that attempts to relate the presence or absence of *Wolbachia* to the sex ratio produced by ant colonies. Chapter 4 investigates the association between mitochondrial haplotypes of hosts and the *Wolbachia* strains they carry. A Swiss population of *F. exsecta* is of particular interest for studying the association between the two maternally inherited elements, because low rates of queen dispersal result in a strong mitochondrial genetic structure between and within ant populations.

Résumé

Cette thèse se propose d'étudier les forces évolutives qui influencent la balance entre coopération et conflit. Deux exemples sont analysés en détail, les sociétés d'insectes et l'association entre la fourmi *Formica exsecta* et son symbiote intracellulaire *Wolbachia*. Chacune de ces deux associations animales est caractérisée par la coexistence de coopération et de conflit.

Les colonies d'insectes sociaux sont un exemple typique de coopération. Néanmoins, la détermination haplodiploïde du sexe crée des apparentements asymétriques entre les membres de la colonie qui se traduisent par des conflits entre reines et ouvrières. Un des conflits les plus importants porte sur le sexe-ratio, l'investissement optimal en sexués males et femelles étant équilibré pour la reine, mais biaisé en faveur des femelles pour les ouvrières. Jusqu'ici, les modèles étudiant ce conflit se basaient uniquement sur les asymétries d'apparentement. La première partie de cette thèse (Chapitres 1 et 2) propose d'introduire dans des modèles de sexe-ratio les mécanismes proximaux utilisés par les deux parties pour influencer le sexe-ratio. Ces mécanismes, par leurs effets négatifs sur la productivité de la colonie, peuvent entraîner des déviations importantes du sexe-ratio par rapport à la valeur prédite par des modèles classiques. De plus, la prise en compte de ces mécanismes nous permet d'étudier dans quelle mesure les parties opposées sont sélectionnées pour influencer le sexe-ratio. Enfin, les modèles génèrent des prédictions quant à l'issue du conflit, c'est-à-dire un compromis où reines et ouvrières partagent le contrôle de l'investissement.

La seconde partie du travail porte sur l'infection de la fourmi *Formica exsecta* par le symbiote cytoplasmique *Wolbachia*. Ce symbiote, présent chez un grand nombre d'espèces d'Arthropodes, est transmis maternellement. Afin de se répandre dans la population d'hôtes, *Wolbachia* manipule la physiologie reproductive de l'organisme qui l'abrite. Le Chapitre 3 décrit une étude consacrée aux variations du sexe-ratio produit dans des colonies de fourmis en fonction de la présence ou de l'absence de *Wolbachia*. Le Chapitre 4 étudie l'association entre les lignées du symbiote et les haplotypes mitochondriaux des hôtes. L'étude de l'association entre les deux éléments maternellement transmis est d'un intérêt particulier chez *Formica exsecta* car le taux de migration femelle très faible engendre une forte structure génétique mitochondriale.

Bibliography

- Aron, S., L. Passera, and L. Keller (1999). Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 266(1415):173–177.
- Backus, V. L. and J. M. Herbers (1992). Sexual allocation ratios in forest ants: food limitation does not explain observed patterns. *Behavioral Ecology and Sociobiology* 30:425–429.
- Boomsma, J. J. (1989). Sex-investment ratios in ants: has female bias been systematically overestimated? *American Naturalist* 133:517–532.
- Boomsma, J. J. and A. Grafen (1990). Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. *Evolution* 44:1026–1034.
- Boomsma, J. J. and A. Grafen (1991). Colony-level sex ratio selection in the eusocial Hymenoptera. *Journal of Evolutionary Biology* 3:383–407.
- Boomsma, J. J., G. A. Van der Lee, and T. M. Van der Have (1982). On the production ecology of *Lasius niger* (Hymenoptera: Formicidae). *Journal of Animal Ecology* 51:975–991.
- Boomsma, J. J., L. Keller, and M. G. Nielsen (1995). A comparative analysis of sex ratio investment parameters in ants. *Functional Ecology* 9(5):743–753.
- Boomsma, J. J., E. J. Fjerdingstad, and J. Frydenberg (1999). Multiple paternity, relatedness and genetic diversity in *Acromyrmex* leaf-cutter ants. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 266(1416):249–254.
- Bourke, A. F. G. (1997). Sociality and kin selection in insects. In Krebs, J. R. and N. B. Davies, editors, *Behavioural Ecology. An Evolutionary Approach. Fourth edition*, pages 203–227. Blackwell, Oxford.

- Bourke, A. F. G. (1999). Colony size, social complexity and reproductive conflict in social insects. *Journal of Evolutionary Biology* 12:245–257.
- Bourke, A. F. G. and G. L. Chan (1999). Queen-worker conflict over sexual production and colony maintenance. *American Naturalist* 154(4):417–426.
- Bourke, A. F. G. and N. R. Franks (1995). *Social evolution in ants*. Princeton University Press, Princeton.
- Bourke, A. F. G. and F. L. W. Ratnieks (1999). Kin conflict over caste determination in social Hymenoptera. *Behavioural Ecology and Sociobiology* 46:287–197.
- Braig, H. R., W. G. Zhou, S. L. Dobson, and S. L. Oneill (1998). Cloning and Characterization of a Gene Encoding the Major Surface Protein of the Bacterial Endosymbiont Wolbachia Pipientis. *Journal of Bacteriology* 180(9):2373–2378.
- Breuer, J. A. J. and J. H. Werren (1990). Cytoplasmic incompatibility and bacterial density in *Nasonia vitripens*. *Genetics* 135:565–574.
- Breuer, J. A. J., R. Stouthamer, S. M. Barns, D. A. Pelletier, W. G. Weisburg, and J. H. Werren (1992). Phylogeny of cytoplasmic incompatibility micro-organisms in the parasitoid wasp *Nasonia* (Hymenoptera: Pteromalidae) based on ribosomal DNA sequences. *Insect Molecular Biology* 1(1):25–36.
- Brian, M. V. (1956). Group form and the cause of working inefficiency in the ant *Myrmica rubra* L. *Physiological Zoology* 29:173–194.
- Brian, M. V. (1979). Habitat differences in sexual production by two co-existent ants. *Anim Ecol* 48:943–953.
- Brian, M. V. (1980). Social control over sex and caste in bees, wasps and ants. *Biological Reviews* 55:379–415.
- Brian, M. V. (1983). *Social Insects: Ecology and Behavioural biology*. Chapman and Hall, London.
- Brown, W. D. and L. Keller (2000). Colony sex ratios vary with queen number but not relatedness asymmetry in the ant *Formica exsecta*. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 267:1751–1757.

- Bulmer, M. and P. D. Taylor (1981). Worker-queen conflict and sex ratio theory in social Hymenoptera. *Heredity* 47:197–207.
- Caspari, E. and G. S. Watson (1959). On the evolutionary importance of cytoplasmic sterility in mosquitoes. *Evolution* 13:568–570.
- Chapuisat, M. and L. Keller (1999). Testing kin selection with sex allocation data in eusocial Hymenoptera. *Heredity*. 82(Part 5):473–478.
- Chapuisat, M., L. Sundström, and L. Keller (1997). Sex-ratio regulation - the economics of fratricide in ants. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 264(1385):1255–1260.
- Charnov, E. L. (1978). Sex-ratio selection in eusocial Hymenoptera. *American Naturalist* 112:317–326.
- Clark, A. B. (1978). Sex ratio and local resource competition in a prosimian primate. *Science* 201:163–165.
- Cook, J. and R. H. Crozier (1995). Sex determination and population biology in the Hymenoptera. *Trends in Ecology and Evolution* 10:281–286.
- Cook, J. M. and R. D. J. Butcher (1999). The transmission and effects of Wolbachia bacteria in parasitoids. *Researches on Population Ecology* 41(1):15–28.
- Crozier, R. H. and P. Pamilo (1993). Sex allocation in social insects: problems in prediction and estimation. In Wrensch, D. L. and M. Ebberts, editors, *Evolution and Diversity of Sex Ratio in Insects and Mites*, pages 369–383. Chapman and Hall, New York.
- Crozier, R. H. and P. Pamilo (1996). *Evolution of social insect colonies. Sex allocation and kin-selection*. Oxford University Press, Oxford.
- Deslippe, R. J. and R. Savolainen (1995). Sex investment in a social insect - the proximate role of food. *Ecology* 76(2):375–382.
- Douglas, A. E. (1996). The biology of symbiotic micro-organisms. *Advances in Ecological Research* 26:69–103.
- Elmes, G. W. (1987). Temporal variation in colony populations of the ant *Myrmica sulcinodis*. 2. Sexual production and sex ratios. *Journal of Animal Ecology* 56:573–583.

- Falconer, D. S. and T. C. F. Mackay (1996). *Introduction to quantitative genetics*. Longman, London, 4th edition.
- Fjerdingstad, E. J., P. J. Gertsch, and L. Keller (2002). Why do some social insect queens mate with several males? Testing the sex-ratio manipulation hypothesis in *Lasius niger*. *Evolution* 56:553–562.
- Fletcher, D. J. C. (1986). Triple action of queen pheromones in the regulation of reproduction in fire ant (*Solenopsis invicta*) colonies. In Porchet, M., J.-C. Andries, and A. Dhainant, editors, *Advances in Invertebrate Reproduction*, volume 4, pages 305–316. Elsevier, Amsterdam.
- Fletcher, D. J. C. and K. G. Ross (1985). Regulation of reproduction in eusocial Hymenoptera. *Annual Review of Entomology* 30:319–343.
- Frank, S. A. (1987). Variable sex ratio among colonies of ants. *Behavioral Ecology and Sociobiology* 20:195–201.
- Frank, S. A. (1998). *Foundations of social evolution*. Princeton University Press, Princeton NJ.
- Giordano, R., S. L. Oneill, and H. M. Robertson (1995). Wolbachia Infections and the Expression of Cytoplasmic Incompatibility in *Drosophila Sechellia* and *D-Mauritiana*. *Genetics* 140(4):1307–1317.
- Grafen, A. (1986). Split sex ratio and the evolutionary origin of eusociality. *Journal of Theoretical Biology* 122:95–121.
- Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology* 7:17–52.
- Hamilton, W. D. (1967). Extraordinary sex ratios. *Science* 156:477–488.
- Hammond, R. L., M. W. Bruford, and A. F. G. Bourke (2002). Ant workers selfishly bias sex ratios by manipulating female development. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 269:173–178.
- Hasegawa, M., H. Kishino, and T. A. Yano (1985). Dating of the Human Ape Splitting by a Molecular Clock of Mitochondrial-DNA. *Journal of Molecular Evolution* 22(2):160–174.
- Helms, K. R. (1999). Colony sex ratios, conflict between queens and workers, and apparent queen control in the ant *Pheidole desertorum*. *Evolution* 53(5):1470–1478.

- Herbers, J. M. (1990). Reproductive investment and allocation ratios for the ant *Leptothorax longispinosus*: sorting out the variation. *American Naturalist* 136:178–208.
- Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner (1999). The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* 14(2):49–53.
- Hoffmann, A. A. and M. Turelli (1997). Cytoplasmic incompatibility in insects. In O'Neill, S. L., A. A. Hoffmann, and J. H. Werren, editors, *Influential Passengers*, pages 42–80. Oxford University Press, Oxford.
- Hoffmann, A. A., D. Clancy, and J. Duncan (1996). Naturally-Occurring Wolbachia Infection in *Drosophila Simulans* That Does Not Cause Cytoplasmic Incompatibility. *Heredity* 76:1–8.
- Hölldobler, B. and S. Bartz (1985). Sociobiology of reproduction in ants. In Hölldobler, B. and M. Lindauer, editors, *Experimental Behavioral Ecology and Sociobiology*, volume 31, pages 237–257. G. Fischer Verlag, Stuttgart.
- Holmes, E. C., M. Worobey, and A. Rambaut (1999). Phylogenetic evidence for recombination in dengue virus. *Molecular Biology and Evolution* 16(3):405–409.
- Hurst, G. D. D., F. M. Jiggins, J. H. G. von der Schulenburg, D. Bertrand, S. A. West, I. Goriacheva, I. A. Zakharov, J. H. Werren, R. Stouthamer, and M. E. N. Majerus (1999). Male-killing Wolbachia in two species of insect. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 266(1420):735–740.
- Hurst, G. D. D., A. P. Johnson, J. H. G. von der Schulenburg, and Y. Fuyama (2000). Male-killing Wolbachia in *Drosophila*: A temperature-sensitive trait with a threshold bacterial density. *Genetics* 156:699–709.
- Jiggins, F. M., J. H. G. von der Schulenburg, G. D. D. Hurst, and M. E. N. Majerus (2001). Recombination confounds interpretations of Wolbachia evolution. *Proceedings of the Royal Society of London Series B-Biological Sciences* 268(1474):1423–1427.
- Keller, L. and M. Chapuisat (1999). Cooperation among selfish individuals in insect societies. *BioScience* 49:899–909.

- Keller, L. and P. Nonacs (1993). The role of queen pheromones in colonies of social insects: Queen control or queen signal? *Animal Behaviour* 45:787–794.
- Keller, L. and H. K. Reeve (1999). Dynamics of conflicts within insect societies. In Keller, L., editor, *Levels of Selection in Evolution*. Princeton University Press, Princeton.
- Kondo, N., N. Ijichi, M. Shimada, and T. Fukatsu (2002). Prevailing triple infection with *Wolbachia* in *Callosobruchus chinensis* (Coleoptera : Bruchidae). *Molecular Ecology* 11(2):167–180.
- Kowalczykowski, S. C. (2000). Initiation of genetic recombination and recombination-dependent replication. *Trends in Biochemical Sciences* 25(4):156–165.
- Lande, R. and S. J. Arnold (1983). The measurement of selection on correlated characters. *Evolution* 37(6):1210–1226.
- Lao, P. J. and D. R. Forsdyke (2000). Crossover hot-spot instigator (Chi) sequences in *Escherichia coli* occupy distinct recombination/transcription islands. *Gene* 243(1-2):47–57.
- Lassy, C. W. and T. L. Karr (1996). Cytological Analysis of Fertilization and Early Embryonic Development in Incompatible Crosses of *Drosophila Simulans*. *Mechanisms of Development* 57(1):47–58.
- Leigh, E. G. (1977). How Does Selection Reconcile Individual Advantage with Good of Group. *Proceedings of the National Academy of Sciences of the United States of America* 74(10):4542–4546.
- Liautard, C. and L. Keller (2001). Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. *Evolution* 55:2484–2492.
- Matessi, C. and I. Eshel (1992). Sex ratio in the social Hymenoptera: a population-genetics study of long-term evolution. *American Naturalist* 139:276–312.
- Maynard Smith, J. and E. Szathmary (1995). *The major transitions in evolution*. Freeman, Oxford.

- Merçot, H., B. Llorente, M. Jacques, A. Atlan, and C. Montchamp-Moreau (1995). Variability Within the Seychelles Cytoplasmic Incompatibility System in *Drosophila Simulans*. *Genetics* 141(3):1015–1023.
- Michener, C. D. (1974). *The social behavior of bees: a comparative study*. Harvard University Press, Cambridge, MA.
- Myers, R. S. and F. W. Stahl (1994). Chi and the Recbcd Enzyme of *Escherichia-Coli*. *Annual Review of Genetics* 28:49–70.
- Nonacs, P. (1986). Ant reproductive strategies and sex allocation theory. *Quarterly Review of Biology* 61:1–21.
- Nonacs, P. (1993). Male parentage and sexual deception in the social Hymenoptera. In Wrensch, D. L. and M. Ebberts, editors, *Evolution and diversity of sex ratio in insects and mites*, pages 384–401. Chapman and Hall, New York.
- Nonacs, P. and N. F. Carlin (1990). When can ants discriminate the sex of brood ? A new aspect of queen-worker conflict. *Proceedings of the National Academy of Sciences of the United States of America* 87:9670–9673.
- Nonacs, P. and J. Tobin (1992). Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera. *Evolution* 46:1605–1620.
- O'Neill, S. L., R. Giordano, A. M. E. Colbert, T. L. Karr, and H. M. Robertson (1992). 16s rRNA Phylogenetic Analysis of the Bacterial Endosymbionts Associated with Cytoplasmic Incompatibility in Insects. *Proceedings of the National Academy of Sciences of the United States of America* 89(7):2699–2702.
- Ortius, D. and J. Heinze (1999). Fertility signaling in queens of a North American ant. *Behavioral Ecology and Sociobiology* 45:151–159.
- Pamilo, P. (1990). Sex allocation and queen-worker conflict in polygynous ants. *Behavioral Ecology and Sociobiology* 27:31–36.
- Pamilo, P. (1991). Evolution of colony characteristics in social insects. 1. Sex allocation. *American Naturalist* 137:83–107.
- Passera, L. (1980). La ponte d'oeufs préorientés chez la fourmi *Pheidole pallidula* (Nyl.) (Hymenoptera: Formicidae). *Insectes Sociaux* 27:79–95.

- Pettis, J. S., H. A. Higo, T. Pankiw, and M. L. Winston (1997). Queen rearing suppression in the honey bee - evidence for a fecundity signal. *Insectes Sociaux* 44:311–322.
- Queller, D. C. (1992). Does population viscosity promote kin selection? *Trends in Ecology and Evolution* 7:322–324.
- Queller, D. C. (2000). Relatedness and the fraternal major transitions. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 355(1403):1647–1655.
- Queller, D. C. and J. E. Strassmann (1998). Kin selection and social insects. *Bioscience* 48(3):165–175.
- Rambaut, A. and N. C. Grassly (1997). Seq-Gen: An application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Computer Applications in the Biosciences* 13(3):235–238.
- Ratnieks, F. L. W. and H. K. Reeve (1992). Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *Journal of Theoretical Biology* 158:33–65.
- Rigaud, T., C. Soutygrosset, R. Raimond, J. P. Mocquard, and P. Juchault (1991). Feminizing Endocytobiosis in the Terrestrial Crustacean *Armadillidium-Vulgare* Latr (Isopoda) - Recent Acquisitions. *Endocytobiosis and Cell Research* 7(3):259–273.
- Rosenheim, J. A., P. Nonacs, and M. Mangel (1996). Sex ratios and multifaceted parental investment. *American Naturalist* 148(3):501–535.
- Rousset, F., D. Vautrin, and M. Solignac (1992a). Molecular Identification of Wolbachia, the Agent of Cytoplasmic Incompatibility in *Drosophila simulans*, and Variability in Relation with Host Mitochondrial Types. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 247(1320):163–168.
- Rousset, F., D. Bouchon, B. Pintureau, P. Juchault, and M. Solignac (1992b). Wolbachia Endosymbionts Responsible for Various Alterations of Sexuality in Arthropods. *Proceedings of the Royal Society of London Series B-Biological Sciences* 250(1328):91–98.

- Sinkins, S. P., H. R. Braig, and S. L. O'Neill (1995). Wolbachia Superinfections and the Expression of Cytoplasmic Incompatibility. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 261(1362):325–330.
- Skinner, S. W. (1982). Maternally inherited sex-ratio in the parasitoid wasp *Nasonia vitripennis*. *Science* 215(4536):1133–1134.
- Stearns, S. C. (1992). *The evolution of life histories*. Oxford University Press, Oxford.
- Stouthamer, R. and D. Kazmer (1994). Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. *Heredity* 73:317–327.
- Stouthamer, R., J. A. J. Breeuwer, R. F. Luck, and J. H. Werren (1993). Molecular-Identification of Microorganisms Associated with Parthenogenesis. *Nature* 361(6407):66–68.
- Sundström, L. (1994). Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. *Nature* 367:266–268.
- Sundström, L. (1995). Sex allocation and colony maintenance in monogyne and polygyne colonies of *Formica truncorum* (Hymenoptera, Formicidae) - the impact of kinship and mating structure. *American Naturalist* 146(2):182–201.
- Sundström, L. and F. L. W. Ratnieks (1998). Sex ratio conflicts, mating frequency, and queen fitness in the ant *Formica truncorum*. *Behavioral Ecology* 9(2):116–121.
- Sundström, L., M. Chapuisat, and L. Keller (1996). Conditional manipulation of sex ratios by ant workers - a test of kin selection theory. *Science* 274(5289):993–995.
- Taylor, P. D. and S. A. Frank (1996). How to Make a Kin Selection Model. *Journal of Theoretical Biology* 180(1):27–37.
- Tracy, R. B., F. Chedin, and S. C. Kowalczykowski (1997). The recombination hot spot *chi* is embedded within islands of preferred DNA pairing sequences in the *E-coli* genome. *Cell* 90(2):205–206.
- Trivers, R. L. and H. Hare (1976). Haplodiploidy and the evolution of the social insects. *Science* 191:249–263.

- Tschinkel, W. R. (1993). Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecological Monographs* 63:427–457.
- Turelli, M. (1994). Evolution of Incompatibility-Inducing Microbes and Their Hosts. *Evolution* 48(5):1500–1513.
- Turelli, M. and A. A. Hoffmann (1991). Rapid Spread of an Inherited Incompatibility Factor in California *Drosophila*. *Nature* 353(6343):440–442.
- Turelli, M. and A. A. Hoffmann (1995). Cytoplasmic Incompatibility in *Drosophila Simulans*: Dynamics and Parameter Estimates From Natural Populations. *Genetics* 140(4):1319–1338.
- Turelli, M., A. A. Hoffmann, and S. W. McKechnie (1992). Dynamics of Cytoplasmic Incompatibility and mtDNA Variation in Natural *Drosophila simulans* Populations. *Genetics* 132(3):713–723.
- van der Have, T. M., J. J. Boomsma, and S. B. J. Menken (1988). Sex-investment ratios and relatedness in the monogynous ant *Lasius niger* (L.). *Evolution* 42:160–172.
- Vargo, E. L. and L. Passera (1991). Pheromonal and behavioral queen control over the production of gynes in the Argentine ant *Iridomyrmex humilis* (Mayr). *Behavioral Ecology and Sociobiology* 28:161–169.
- Vavre, F., F. Fleury, D. Lepetit, P. Fouillet, and M. Bouletreau (1999). Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. *Molecular Biology and Evolution* 16(12):1711–1723.
- Vavre, F., F. Fleury, J. Varaldi, P. Fouillet, and M. Bouletreau (2000). Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: Epidemiologic and evolutionary consequences. *Evolution* 54(1):191–200.
- von der Schulenburg, J. H. G., G. D. D. Hurst, T. M. E. Huigens, M. M. M. van Meer, F. M. Jiggins, and M. E. N. Majerus (2000). Molecular evolution and phylogenetic utility of *Wolbachia* *ftsZ* and *wsp* gene sequences with special reference to the origin of male-killing. *Molecular Biology and Evolution* 17(4):584–600.
- Wenseleers, T. and J. Billen (2000). No evidence for *Wolbachia*-induced parthenogenesis in the social Hymenoptera. *Journal of Evolutionary Biology* 13(2):277–280.

- Wenseleers, T., F. Ito, S. Vanborm, R. Huybrechts, F. Volckaert, and J. Billen (1998). Widespread Occurrence of the Micro-Organism *Wolbachia* in Ants. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 265(1404):1447–1452.
- Wenseleers, T., L. Sundstrom, and J. Billen (2002). Deleterious *Wolbachia* in the ant *Formica truncorum*. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 269(1441):623–629.
- Werren, J. H. (1997). Biology of *Wolbachia*. *Annual Review of Entomology* 42:587–609.
- Werren, J. H. and J. D. Bartos (2001). Recombination in *Wolbachia*. *Current Biology* 11(6):431–435.
- Werren, J. H. and S. L. O'Neill (1997). The evolution of heritable symbionts. In O'Neill, S. L., A. A. Hoffmann, and J. H. Werren, editors, *Influential Passengers*, pages 1–41. Oxford University Press, Oxford.
- Werren, J. H., D. Windsor, and L. R. Guo (1995a). Distribution of *Wolbachia* Among Neotropical Arthropods. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 262(1364):197–204.
- Werren, J. H., W. Zhang, and L. R. Guo (1995b). Evolution and Phylogeny of *Wolbachia*: Reproductive Parasites of Arthropods. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 261(1360):55–63.
- Wheeler, D. E. (1986). Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *American Naturalist* 128:13–34.
- Zhou, W. G., F. Rousset, and S. Oneill (1998). Phylogeny and PCR-based Classification Of *Wolbachia* Strains Using *wsp* Gene Sequences. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 265(1395):509–515.