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Caste determination through mating in primitively eusocial societies

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HIGHLIGHTS

- In primitively eusocial insects, it is often unclear how caste is determined.
- We investigate the evolutionary stability of caste determination by mating status.
- The model fits field observations if early workers are more valuable than late ones.
- High male mortality and low worker mortality make this fit more likely.
- High worker value and a long period over which workers help also make it more likely.

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ABSTRACT

Eusocial animal societies are typified by the presence of a helper (worker) caste which predominantly cares for young offspring in a social group while investing little in their own direct reproduction. A key question is what determines whether an individual becomes a worker or leaves to initiate her own reproduction. In some insects, caste is determined nutritionally during development. In others, and in vertebrate societies, adults are totipotent and the cues that determine caste are less well known. The mate limitation hypothesis (MLH) states that a female's mating status acts as a cue for caste determination: females that mate become reproductives, while those that fail to mate become workers. The MLH is consistent with empirical observations in sweat bees showing that over the course of the nesting season, there are increases in both the proportion of females that become reproductives and the frequency of males in the mating pool. We modelled a foundress's offspring sex-ratio strategy to investigate whether an increasingly male-biased operational sex-ratio over time is evolutionarily stable under the MLH. Our results indicate that such a pattern could occur if early workers were more valuable than late workers. This pattern was then more likely if male mortality was high, if worker mortality was low, if the value of a worker was high and if the period over which workers can help was short. Our results suggest that the MLH can be evolutionarily stable, but only under restrictive conditions. Manipulative experiments are now required to investigate whether mating determines caste in nature.

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1. Introduction

One of the key questions about social evolution concerns caste determination: what determines whether an individual chooses to become a helper (worker) within its natal group or leaves to initiate its own reproduction as a breeder (queen)? Since Hamilton (Hamilton, 1964), a large body of work has focussed on the functional aspects of this question: under what conditions are genetic relatedness, and the benefit/cost ratio, high enough to promote helping? There has been less emphasis, however, on the

mechanistic aspect: what cues or signals do individuals use in making these decisions? In so-called 'highly eusocial' insects such as ants and honeybees, female caste is determined during immature development. For example, adult workers may lack functional spermathecae, so that they cannot mate. Caste is then thought to usually be determined through the quantity and quality of food provided for immature larvae, although instances of genetically based determination have recently been highlighted (Schwander et al., 2010).

By contrast, in so-called 'primitively eusocial' species, such as many wasps and bees, adults are totipotent and are thought to choose what strategy to follow based on information about their own phenotypes and the social and physical environment. Thus, newly emerged adult females in temperate regions might use cues associated with time of year and their own condition when

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deciding whether to immediately start helping in their natal groups or enter hibernation and initiate new nests as reproductives the following year (e.g. Reeve et al., 1998; Hunt et al., 2010; Field et al., 2010; West-Eberhard, 2003). Parents and other nest-mates could also influence decision-making, for example by inducing 'subfertility' in offspring (West-Eberhard, 1975; Craig, 1983; Linksvayer and Wade, 2005). Additionally, recent work on a polistine wasp suggests that adult females provide direct signals that developing larvae, or the adults feeding them, use to determine offspring caste (Suryanarayanan et al., 2011).

In this paper, we focus on the feasibility of another potential cue for caste determination: mating status. Yanega (1989, 1997) suggested that whether a female becomes a helper depends on whether she mates within a short time of reaching adulthood. We will follow Yanega in referring to this as the 'mate limitation hypothesis' (MLH). The empirical observations that led (Yanega, 1989, 1997) to formulate the MLH were made on sweat bees (*Halictidae*). Sweat bees are especially valuable for understanding the genetic and environmental mechanisms underlying the origins of eusociality because, unlike nearly all other hymenopteran lineages: (1) there are closely related eusocial and non-social ('solitary') sweat bee species; (2) there is evidence of repeated switching between eusociality and non-sociality during sweat bee evolution and (3) there are socially polymorphic sweat bees where females live in social groups in some populations, but are solitary in other populations of the same species; or both social and non-social nests exist in the same population (Field et al., 2010, 2012; Gibbs, 2012; Yagi and Hasegawa, 2010).

We assume a life history (Fig. 1), documented in Yanega's data (Yanega, 1989), that is probably typical of many temperate primitively eusocial wasps and bees, such as many sweat bees (*Halictus*, *Lasio-glossum*) and paper wasps (*Polistes*). In spring, each overwintered, mated female, known as a foundress, constructs a separate nest where she alone rears a first brood (B1) of offspring of both sexes. When the B1 females mature, some mate with B1 males then overwinter to become foundresses the following year (e.g. Reeve et al., 1998; Yanega, 1989). Other B1 females, however, become helpers which forage to provision a second brood (B2) of male and female offspring. These offspring are produced by the foundress if she is still alive, or by one of the B1 females if the foundress is dead (e.g. Field et al., 2010; Yanega, 1989) (Fig. 1). After mating, all B2 females overwinter, but helpers never subsequently overwinter (e.g. Yanega, 1989). Males die before winter and are not involved in nesting.

We focus on factors that determine whether B1 females become helpers or overwinter and become reproductives the following year. According to the MLH, females that mate during

the critical period enter hibernation whereas females that do not mate become workers (but do not lose the ability to mate subsequently). In his detailed study of *Halictus rubicundus*, Yanega (1989) observed that during the period when B1 offspring mature to adulthood: (1) the probability that a B1 female will enter hibernation is greater for late-maturing females than early-maturers, gradually increasing until late in the season when all females hibernate; (2) the sex-ratio of offspring in the mating pool appears to become gradually more male-biased. Thus, an increasing probability of entering hibernation is associated with an increasingly male-biased operational sex ratio (OSR) and therefore presumably with an increased chance that female offspring will mate promptly after reaching adulthood. *H. rubicundus* is socially polymorphic and the B1 sex-ratio is more male-biased in solitary than social populations (Yanega, 1997; Soucy, 2002). Thus, the MLH can also potentially explain the existence of univoltine solitary populations, in which all B1 females enter hibernation and none become workers (Soucy, 2002). Note that these arguments contrast with ideas regarding the sex-ratio during the B2 period, where an increasingly female-biased sex ratio is expected (Richards et al., 1995) and has been observed (Yanega, 1989). The main difference between these cases is that in the B2 period workers are not produced, thus changing the potential value of females relative to males.

Although the MLH is often referred to in studies of sweat bees (e.g. Soucy, 2002; Plateaux-Quenu and Packer, 1998; Hirata and Higashi, 2008; Soro et al., 2010; Soucy and Danforth, 2002), Yanega's (1989) data were only correlative. Furthermore, the evolutionary stability of the MLH seems questionable, as follows. Under the MLH, the observed pattern, whereby late-maturing B1 females are more likely to mate, relies on the OSR becoming increasingly male-biased as the maturation period progresses. This could be driven by the foundresses that produce the B1 generation, if they produce a sex-ratio that is initially female-biased but gradually becomes more male-biased. Effectively, the options of B1 offspring would be constrained by the sex-ratio strategy of foundresses. But what is then to stop some foundresses from 'cheating' by producing early males to take advantage of early females that would otherwise fail to mate and so become workers under the MLH? If mating directly determines caste, matings by such early males would cause early females to become reproductives the following year, thus transmitting the early males' genes. In this paper, we aim to model temporal changes in the OSR under the MLH. In particular, we determine whether or not, under the MLH, there are evolutionarily stable conditions where the mating pool sex-ratio does become gradually more male-biased over time, consistent with Yanega's (1989) observations and interpretation. In other words, we test whether Yanega's field observations are consistent with his hypothesis.

2. Methods

We use an Evolutionary Stable Strategy (ESS) model in which we build fitness equations for the sex-ratio strategies of foundresses under Yanega's (1989, 1997) 'mate limitation hypothesis' (MLH). Thus, we assume that whether a B1 female becomes a worker depends on whether she mates soon after reaching adulthood. Workers help to produce a second generation (B2) whose females all enter hibernation. The parameters and symbols used in this section and in the results are summarised in Table 1.

2.1. The model

2.1.1. An individual's strategy

For mathematical convenience, we divide the B1 brood rearing period into T successive time steps and assume that foundresses

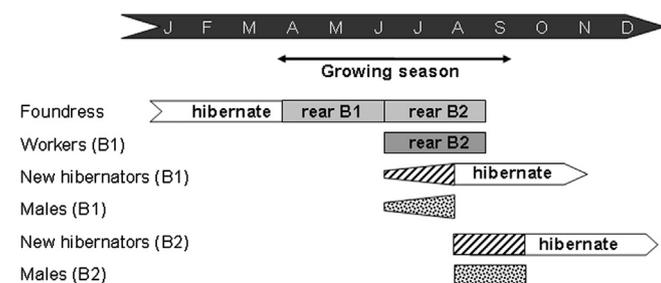


Fig. 1. The life cycle of colonies in our model. At the beginning of the season, hibernating females born the previous year, known as foundresses, each start a new colony and alone raise an initial brood (B1) consisting of males, workers, and hibernators: females that will enter hibernation to become foundresses the following year. Workers help the foundress to rear a second brood (B2) consisting of further males and hibernators. Increasing width of bars representing males indicates increasing male frequency in the mating pool, as found by Yanega (1989) in *Halictus rubicundus*. Increasing width of bars representing new hibernators indicates an increasing proportion of females becoming hibernators rather than workers. Modified from Fig. 1 in Chapuisat (2010).

Table 1
Definition of the parameters and symbols.

Value name	Definition	Definition in model with worker mortality (if different)
a	Value of a worker relative to a B1 hibernator	Value of a worker, relative to a B1 hibernator, per time step that the worker remains alive
b	Fitness obtained from B2 hibernators in the absence of workers	Fitness obtained from B2 hibernators in one time step in the absence of workers
F_t	Number of females in the mating pool at time t	
m	Male mortality rate	
M_t	Number of males in the mating pool at time t	
m_ω	NA	Worker mortality rate
n	Number of foundresses in the population	
p	Number of brood produced by a given foundress	
PFM	Proportion of females in the mating-pool for a given solution to the model	
s_t, \mathbf{s}	Population mean brood-production sex ratio at time t , and vector of all values of s_t respectively*	
T	Number of time steps in the B1 brood emergence period	
T_ω	NA	Number of time steps in the B2 brood-rearing period (the max number of time steps over which a worker emerging on the first time step can help)
W_i	Total fitness of focal foundress	
W_{h_i}, W_{M_i}	Fitness of focal foundress from B1 hibernators and B1 males respectively	
W_{ω_i}	Fitness of focal foundress from the B2 brood (obtained through B1 workers)	
X_t	Sex ratio at time t , expressed as M_t/F_t	
α_t	Probability that a female will mate at time t	
β	Slope through the ranked values of PFM against time	
ω_i	Total number of workers produced by the focal foundress	

* Subscript i indicates a value for a focal female instead of a population mean.

can control the sex ratio of their brood at each time step independently. In the main text, we primarily model two time steps ($T=2$). These two steps should not be confused with the two different brood-rearing periods (B1 and B2).

A foundress's brood sex-ratio strategy for the B1 phase thus consists of T sex-ratios (proportion of brood that are female on each time step). Specifically, the i th foundress's strategy is defined by the vector $\mathbf{s}_i = (s_{i1}, s_{i2}, \dots, s_{iT})$, such that on any given time step t , the foundress produces a proportion s_{it} of females ($0 \leq s_{it} \leq 1$). In other words, s_{it} is the i th foundress's brood sex ratio at time step t .

2.1.2. The population

The population as a whole produces the sex ratio $\mathbf{s} = (s_1, s_2, \dots, s_T)$, where s_t is the population mean of s_{it} (we assume that all strategies produce the same number of offspring). The population is assumed to be sufficiently large that \mathbf{s} is not affected by a focal foundress's value of \mathbf{s}_i .

The sex ratio we are primarily interested in is that in the B1 mating pool. The MLH suggests that B1 females disappear from the mating pool relatively rapidly, either because they fail to mate and so become workers, or because they mate and enter hibernation. Therefore, in our model, females do not leave the mating pool due to mortality. Males, on the other hand, continue to form part of the mating pool until they die or until time T , when all B1 females have reached adulthood, so that no further matings are possible. We assume that B1 males do not survive long enough to mate with B2 females.

We assume that females can mate only on the time step at which they reach adulthood. Therefore, the number of females in the mating pool at time t is

$$F_t = s_t np \tag{1}$$

where n is the number of foundresses in the population and p is the number offspring produced by a foundress each time step.

The number of males produced in a given time step is

$$\Phi_t = (1-s_t)np \tag{2}$$

However, males that have survived from previous time steps will also be present. If m is the mortality of males from one time step to the next, then the number of males present in the mating pool at time t is

$$M_t = \sum_{j=1}^t [\Phi_j (1-m)^{t-j}] = \sum_{j=1}^t [(1-s_j)np(1-m)^{t-j}] \tag{3}$$

Note that if $m = 1$ then the number of males in the mating pool at a given time step is simply the number that reach adulthood in that time step, since no males survive between steps.

The sex ratio X in the mating pool at time t is M_t/F_t , that is

$$X_t = \frac{\sum_{j=1}^t [(1-s_j)np(1-m)^{t-j}]}{s_t np} \tag{4}$$

2.1.3. Mating probability

The probability that a female will mate on a given time step (α_t) is a function of the number of males and females in the mating pool at that time, that is a function of X_t . We assume that α_t should increase as X_t increases and that this increase should follow a curve of diminishing returns (an extra male when male numbers are very small will have a larger effect than when males are already very common). We also assume that when the sex ratio is 1:1, most females will mate successfully. Finally, we stipulate that when there are no males in the population, the probability that a female will mate must be 0, and that when males are rare, $\alpha_t > X_t$ (implying that males are capable of multiple mating). These properties can be produced by an exponential function such as

$$\alpha_t = 1 - e^{-3X_t}, \tag{5}$$

or a hyperbolic function such as

$$\alpha_t = 1 - \frac{1}{5X_t + 1}, \tag{6}$$

These are standard curves of diminishing returns with parameters chosen manually to fulfil the above assumptions.

2.1.4. Fitness from female production

We define the fitness of a female that goes into hibernation as 1. This includes the chance of mortality during the hibernation period. The fitness that a foundress gains at time t from producing hibernating females is equal to the number of females she produces at time t multiplied by the probability that a given female will mate and therefore enter hibernation under the MLH. The i th foundress's fitness from B1 hibernators across all time steps is therefore

$$W_{hi} = \sum_{t=1}^T s_i p \alpha_t \tag{7}$$

If she fails to mate (with probability $1-\alpha$), a female becomes a worker, so that the number of B1 workers which a foundress produces is

$$\omega_i = \sum_{t=1}^T s_i p (1-\alpha_t) \tag{8}$$

We have now obtained values for the number of hibernators, workers and males which a foundress can expect to produce over the course of the B1 period. Subsequent to this, further hibernators and males will be produced in the B2 period. We assume that a foundress's fitness from the B2 brood will depend on the number of workers which she has produced in the B1 period, and on how many individuals a worker can expect to rear in the B2 brood. For now, we summarise all the benefits that workers can provide to the nest in a single value W_{ω_i} , which we define as the fitness that a foundress obtains through B2 brood, and which is an increasing function of ω_i . Indeed, a larger number of workers normally leads to larger total B2 productivity (e.g. Yanega, 1989; Strohm and Bordon-Hauser, 2003).

2.1.5. Fitness from male production

A foundress's fitness from B1 males (W_{M_i}) depends on how many hibernating females her sons successfully mate with. We assume that each successful mating on the part of her sons is worth as much to a foundress as a daughter that enters hibernation (our standard of 1). If we assume that each B1 female mates only once, then the number of B1 females that a son can expect to mate with at time t is the number of females which will mate at time t divided by the number of males present at time t , that is $F_t \alpha_t / M_t$. Taking into account the fact that these sons can also survive to mate in future time steps, the overall fitness that a foundress gets from the males she produces at time t is

$$W_{\phi_i}(t) = \sum_{k=0}^{T-t} \left[(1-s_i) p (1-m)^k \frac{F_{t+k} \alpha_{t+k}}{M_{t+k}} \right] \tag{9}$$

Most male bees and wasps can mate repeatedly (Shilpa et al., 2012; Paxton, 2005), and males can certainly survive beyond their date of birth (e.g. J.F unpublished observations of marked male *H. rubicundus*), so that early-produced males are indeed likely to be more successful than late-produced ones, who will find themselves surviving beyond the end of the period of female emergence. The overall fitness of a foundress from males is therefore

$$W_{M_i} = \sum_{t=1}^T W_{\phi_i}(t) = \sum_{t=1}^T \sum_{k=0}^{T-t} \left[(1-s_i) p (1-m)^k \frac{F_{t+k} \alpha_{t+k}}{M_{t+k}} \right] \tag{10}$$

2.1.6. Overall fitness

A female's overall fitness is the sum of her fitness from B1 hibernators, B2 brood and B1 males:

$$W_i = W_{hi} + W_{\omega_i} + W_{M_i} \\ = \left(\sum_{t=1}^T s_i p \alpha_t \right) + W_{\omega_i} + \sum_{t=1}^T \sum_{k=0}^{T-t} \left[(1-s_i) p (1-m)^k \frac{F_{t+k} \alpha_{t+k}}{M_{t+k}} \right], \tag{11}$$

If we substitute the terms that are functions of s_i and s (except α and W_{ω_i} , which we leave undefined for now) we get

$$W_i = \left(\sum_{t=1}^T s_i p \alpha_t \right) + W_{\omega_i} \\ + \sum_{t=1}^T \sum_{k=0}^{T-t} \left[(1-s_i) p (1-m)^k \frac{s_{t+k} \alpha_{t+k}}{\sum_{j=1}^{t+k} [(1-s_j)(1-m)^{t+k-j}]} \right] \tag{12}$$

Because the sex ratio produced by one foundress will not significantly affect the sex ratio of the population, the fitness that a foundress obtains from each offspring of a given sex is independent of the brood sex ratio she produces. Eqs. (7) and (9) implicitly assume that the fitness which a foundress obtains from hibernators (and males) is a linear function of the number of hibernators (and males) she has produced. That is, each reproductive offspring of a given sex is worth as much as any other, irrespective of the total number that the foundress produces. If we assume that the value of workers, in the form of offspring in the B2 generation that workers help to rear, is a linear function of the number of workers (i.e. each worker in a nest is worth as much as the last), then the fitness obtained by a foundress via B2 brood is

$$W_{\omega_i} = a \omega_i + b \\ = a \left(\sum_{t=1}^T s_i p (1-\alpha_t) \right) + b \tag{13}$$

where a and b are the slope and intercept of the linear relationship. b can be seen as the fitness that a foundress obtains from B2 offspring in the absence of workers, while a is the fitness added by each worker. a can therefore be seen as the value of a worker relative to a B1 hibernator.

In this case, because W_{hi} , W_{ω_i} and W_{M_i} are all linear functions of s_i , W_i will also be a linear function of s_i and $\partial W_i / \partial s_i$ will be independent of s_i . In order to determine what temporal patterns the MLH leads to in the OSR, we seek to solve $\partial W_i / \partial s_i = 0$, that is the t simultaneous equations:

$$\frac{\partial W_i}{\partial s_i} = 0 \tag{14}$$

As s_i has disappeared from the differentials, all strategies will have the same fitness when the equilibrium conditions are satisfied. This is a similar situation to classical sex ratio theory, which shows that the stable Environmental Sex Ratio (ESR) is always 1:1, but that all strategies can co-exist so long as the ESR is maintained.

2.2. Solving the model

Consider a simple version of the model in which there are only two time steps ($T=2$). The first time step then represents 'early' B1 offspring; the second time step represents 'late' offspring. The differential equations are then

$$\begin{cases} \frac{\partial W_i}{\partial s_1} = p \left[\alpha_1 + a(1-\alpha_1) - \frac{s_1}{1-s_1} \alpha_1 + (1-m) \frac{s_2}{(1-s_1)(1-m)+(1-s_2)} \alpha_2 \right] \\ \frac{\partial W_i}{\partial s_2} = p \left[\alpha_2 + a(1-\alpha_2) - \frac{s_2}{(1-s_1)(1-m)+(1-s_2)} \alpha_2 \right] \end{cases} \tag{15}$$

The values of s_1 (the sex-ratio of early offspring) and s_2 (the sex-ratio of late offspring) will change over evolutionary time as a response to natural selection on sex ratio strategy. We therefore seek the values of s_1 and s_2 in which Eq. (15) are simultaneously equal to 0.

b and n have disappeared from (15), and it can be seen that p will do the same when Eq. (15) are set to 0. We are therefore left with m (male mortality) and a (the incremental value of workers) as the parameters that could affect the solutions to the model. This remains the case when $T > 2$.

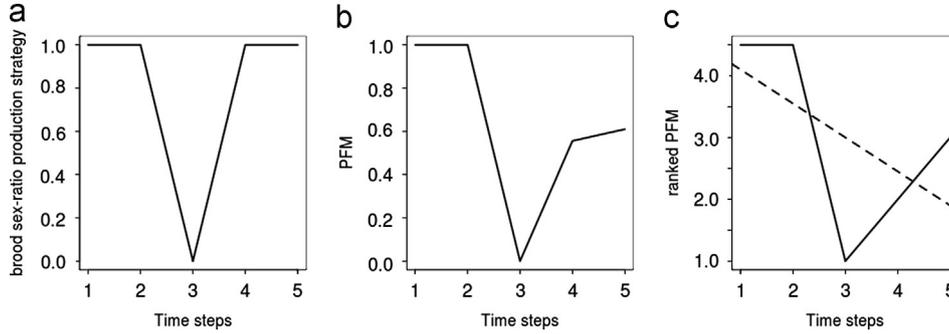


Fig. 2. Illustration of the procedure used to interpret the results of the model. In this case, the model was run with five time steps ($T=5$), generating a brood sex-ratio production strategy (the proportion of larvae reaching adulthood that are females) at each step (a). Using this solution, the proportion of females in the mating pool (PFM) at each time step could be calculated, bearing in mind that males can remain in the mating pool for more than one time step (in this example, $m=0.2$, i.e. 80% of males survive from one step to the next), whereas females always exit at the end of the time step in which they reached adulthood (b). These PFM values were ranked and the slope (β) of these points against time was calculated (c). The plots in this figure were generated from one of the four solutions obtained from the model with additional parameter values $a=10$ and with the value of workers independent of the time step when they mature.

The system of differential equations was solved numerically using the open source mathematical software package SAGE (v. 2.6.1, www.sagemath.org). We used iterative methods that required an initial value of \mathbf{s} to be provided (see Online Resources 1 for more details). For a given set of parameter values, we searched for solutions with a range of initial guesses (Online Resources 1) in order to identify the existence of multiple solutions (local optima).

2.3. Solving the differential equations when worker value is dependent on emergence time

The above model implicitly assumes that workers have the same value, regardless of the time step at which they reach adulthood. We can relax this assumption by including a term for worker mortality (m_w) and a term for the number of time steps after which the B2 brood-rearing period ends ($T_w; T_w \geq T$). A female reaching adulthood and becoming a worker at time t can therefore help, but also suffers mortality, for up to $T_w - t$ time steps. This means that early-maturing workers can potentially help for longer than late-maturing ones and so help to produce more B2 offspring. *A priori*, this scenario would seem to have the potential to select for an initially female-biased offspring sex ratio, as observed by Yanega (1989).

Here a is defined as the productivity of each worker at each time step during which it survives, which is again assumed to be constant irrespective of the number of workers present in the nest. The overall value of a worker that emerges at time t is therefore

$$a \sum_{k=1}^{T_w-t+1} (1-m_w)^k \tag{16}$$

Note that this implies that workers pass an initial round of mortality before beginning to work. The overall fitness that a given foundress gets from workers is

$$W_{w_i} = \sum_{t=1}^T \left[s_{i_t} p(1-\alpha_t) a \left(\sum_{k=1}^{T_w-t+1} (1-m_w)^k \right) + b \right] \tag{17}$$

With $T=2$, this gives us

$$\frac{\partial W_{w_i}}{\partial s_{i_1}} = ap(1-\alpha_1) \left(\sum_{k=1}^{T_w} (1-m_w)^k \right) \tag{18}$$

and

$$\frac{\partial W_{w_i}}{\partial s_{i_2}} = ap(1-\alpha_2) \left(\sum_{k=1}^{T_w-1} (1-m_w)^k \right) \tag{19}$$

Whereas the only terms that could affect the solutions to Eq. (15) were m and a , here we have two extra terms that need to be considered: m_w (the mortality rate of workers) and T_w (the number of steps available in which to produce B2 offspring). These equations were again solved in SAGE using the same methods as described above.

2.4. Number of time steps

The model was initially solved with two time steps ($T=2$). To examine whether the results from the model with two time steps were consistent when the number of time steps was increased, the model was also run with 5 time steps ($T=5$).

3. Results

For a given set of parameter values, the solution to the model was a strategy consisting of a stable, population-wide B1 brood-production sex ratio for each time step. For a simulation run with T time steps, the result therefore consists of T values. For each production sex ratio strategy, we can also calculate the resulting proportion of females in the mating pool (PFM) at each time step. To summarise these T values of PFM as a single value, and to better illustrate any temporal trend graphically, we ranked them then calculated the slope through the ranks (β) against time ($-1 < \beta < 1$). This process is illustrated in Fig. 2. β can therefore be seen as the extent to which the female-bias of the mating pool tends to become more female-biased with time. A positive β indicates that the mating pool tends to become more female-biased with time. A negative β indicates that the mating pool tends to become less female-biased over time, as observed by Yanega, 1989). Because both the x and y values of this regression range from 1 to T , β must always lie between -1 and 1 . For results from the two time steps model, β can of course be only 1, 0 or -1 . Here we present results using the hyperbolic equation for α (Eq. (6), where α is a female's probability of mating, given the sex ratio). Results using Eq. (5) are qualitatively similar, though some small differences existed (Online Resources 2).

3.1. Model with two time steps

The results of the model run with two time steps are shown in Figs. 3 and 4. It should be noted that some of the parameter combinations represented in Fig. 4c and d generated multiple solutions. However, this occurred in only a few cases, and the alternative solutions did not change the trends discussed below.

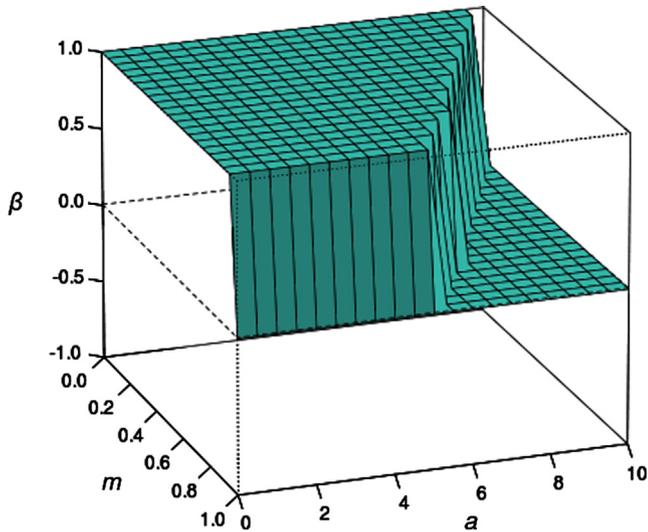


Fig. 3. Surface plot showing the relationship between male mortality (m), the relative value of workers (a) and the slope (β) of the ranked PFM against time (see Fig. 2) in the two time-step model where all workers have the same value. A negative slope (β) indicates that the mating pool becomes more male-biased over time, as observed by Yanega (1989). The results indicate that such an increase in male-bias over time was never possible. We note that some parameter combinations produced two solutions (though only one is represented here). These always occurred at points that were on the boundary between the $\beta = -1$ and $\beta = 1$ planes and that had a solution on each of these planes.

We therefore represent only one solution graphically for each of these cases.

We first examine the model where all workers had the same value regardless of their emergence times. Under these conditions, a lower PFM in the second time step compared to the first never occurred (Fig. 3). Either the PFM was higher in the second time step (i.e., $\beta > 0$, the mating pool becomes more female-biased with time) or it was the same on both time steps ($\beta = 0$). A PFM equal at both time steps occurred when workers had such high value that no males were produced on either time step (PFM = 1), leading to a B1 brood consisting entirely of workers (since none of the females could mate). Hibernators are then produced only in the B2 generation. This tended to be the case when worker value (a) and male mortality (m) were high. The only other conditions under which the PFM was equal at both time steps occurred when male mortality was equal to 1; this is because all time steps effectively become completely independent in this case and the optimal strategy must therefore be the same for all steps. It thus appears that, when workers all have the same value, the MLH cannot lead to the temporal increase in the proportion of males in the mating pool reported by Yanega (1989).

In the model where early workers could be more valuable than late ones, however, both increases and decreases in PFM with time were possible. There tended to be a lower PFM in the second time step relative to the first ($\beta < 0$, that is a decrease in the mating pool's female bias with time) when male mortality (m) was high (Fig. 4a and b), when worker mortality (m_w) was low (Fig. 4c and

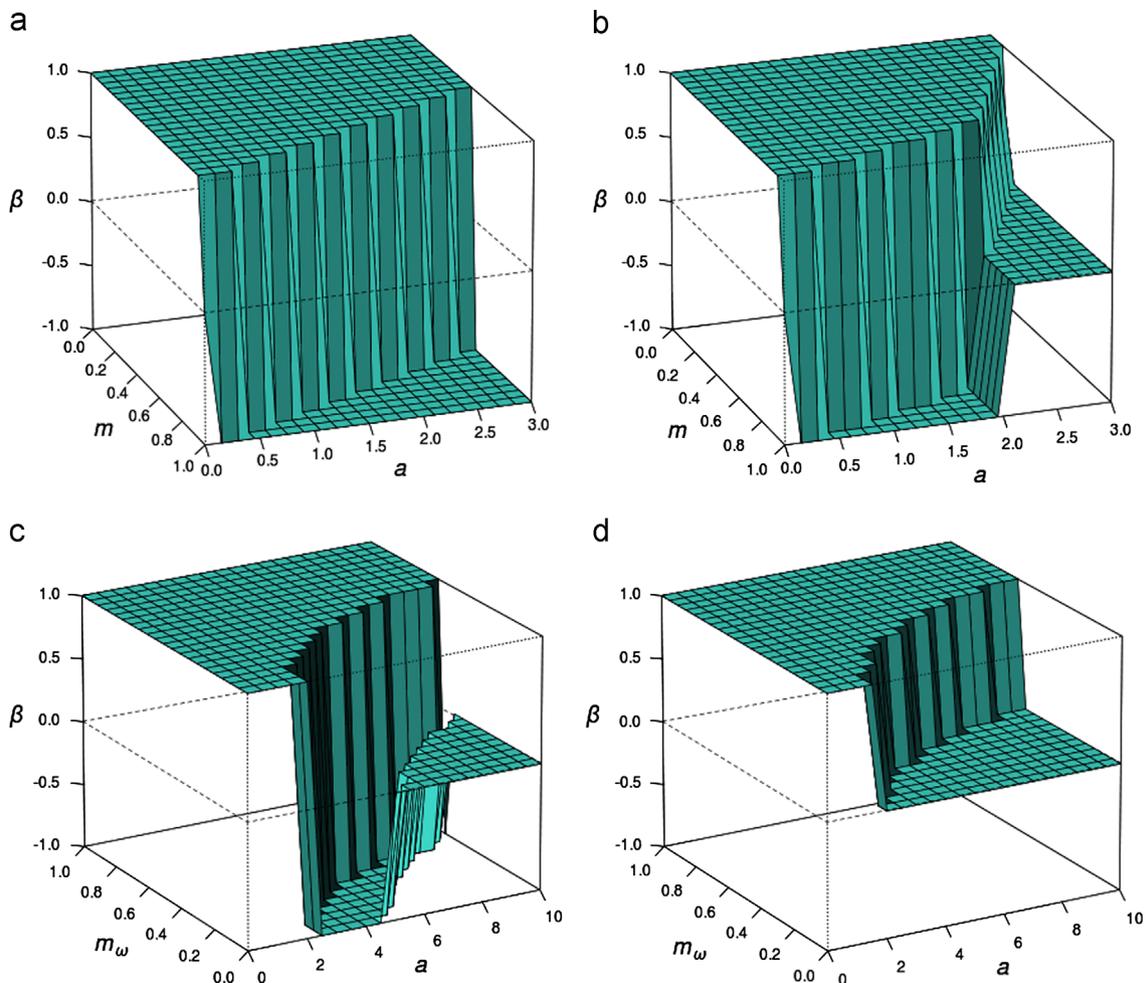


Fig. 4. Surface plots showing the relationship between male mortality (m , a and b) or worker mortality (m_w , c and d), the relative value of workers (a) and the slope (β) of the ranked PFM against time in the two time step model. We note that the range of the a axis differs between plots and that the m_w axis in (c) and (d) is reversed compared with the m axis in (a) and (b). (a) $T_w = 2$, $m_w = 0.1$. (b) $T_w = 4$, $m_w = 0.1$. (c) $T_w = 2$, $m = 0.6$. (d) $T_w = 4$, $m = 0.6$.

d), and when the value of a worker (a) was high (Fig. 4a–d). As male mortality increases, the relative value of an early male decreases and early females therefore become relatively more valuable. Similarly, as worker mortality decreases, the relative value of early females increases (because those that become workers will suffer lower mortality). As the value of workers increases, the proportion of females in the mating pool increases at both time steps, but does so more strongly for the first time step than the second when β is close to 0 (Online Resources 3). Increasing the value of workers will therefore tend to favour a decrease in the proportion of females in the mating pool with time.

As in the model where early and late workers are equally valuable, exclusive worker production can occur on both time steps if workers have high value relative to foundresses or males (if the time over which workers can help (T_w) is high, male mortality (m) is high or worker value (a) is high). This leads to an apparent reversal of the result that β decreases with increasing worker value (a), because as a increases, β changes from -1 to 0 (from a decreasing female-bias to no change in the female bias with time) (Fig. 4b). In fact, it may still be the case that selection favours females at early time steps even more strongly than in later steps, but the biological limit of 1 imposed on PFM leads to no change in the PFM with time because PFM is equal to one at all time steps (exclusively worker production).

When there are more time steps during which workers can help to produce B2 offspring (larger T_w) then there is less chance of obtaining a negative β , i.e. a temporal decrease in the proportion of females in the mating pool of the kind observed by Yanega (1989) (Fig. 4). This occurs because extending the number of time steps for which workers can help makes late workers almost as valuable as early workers. For example, taking the case where worker mortality is 0.5, if workers can help for two time steps then an early worker would be 1.5 times as valuable as a late worker, whereas if workers can help for 10 time steps, an early worker would have around the same value as a late worker. It is perhaps surprising that this effect is not stronger. An increase in T_w also leads to a large increase in the chances of finding exclusive worker production in both time steps (Fig. 4).

3.2. Model with multiple time steps

When run with multiple time steps, a much greater number of parameter combinations generated several solutions, and the trends of β against the various parameters were not monotonic. This makes it hard to draw general conclusions from the results, although the apparent trends are similar to those obtained from the two time-step model (see Online Resources 2 for more details). Solutions with negative β (a decrease in the female-bias of the mating pool with time) were possible for many parameter combinations in which worker value was very high ($a \geq 5$). These solutions were mostly strategies of producing an initial 100% female brood on the first time step or the first two time steps, which led to an overall negative slope for PFM, but never a monotonic decrease in PFM over time (e.g., see the example depicted in Fig. 2).

An interesting feature of many of the multiple solutions is that they consist of producing exclusively one sex on some time steps, and exclusively the other sex on other time steps, while the order in which the sexes are produced can vary between solutions found with the same combination of parameter values (Fig. 5). It seems that populations can become “locked” into producing a certain sex at a certain time step, because producing that sex is advantageous even when all individuals are producing it, so that a population sex ratio of 100% of either sex at a given time step is stable, and a variety of “locked” states can then exist from one time step to the next. In nature, however, this is unlikely to occur as it requires that

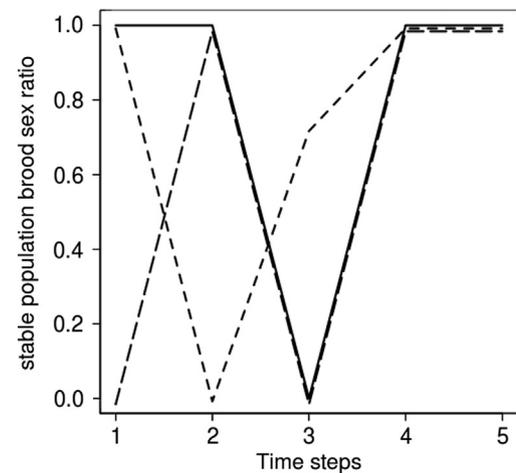


Fig. 5. Three possible solutions to the model with the following parameter values: $m=0.3$, $m_w=0.2$, $a=3$, $T=5$, $T_w=10$, showing the variability of solutions that can emerge. The lines represent the stable proportion of females in the brood reaching adulthood (averaged over the population) at each time step. Points are shifted slightly vertically in order to show overlapping lines.

the offspring sex ratio be precisely controlled on each day that offspring reach adulthood. As brood reach adulthood several weeks after the eggs are laid, and as foundresses are unlikely to have perfect control over the developmental period of each offspring, we consider that random effects on brood sex ratio timing would prevent these solutions of fluctuating extremes. The model therefore fails to provide an adequate simulation of reality when broken down into so many time steps, and we consider it more informative to focus on the results of the two time step model, which simulates a much coarser scenario.

4. Discussion

The hypothesis that mating determines caste in primitively eusocial Hymenoptera has been best developed by Yanega (1989, 1997). Yanega's hypothesis derived from his detailed demographic data on the sweat bee *H. rubicundus*, but could apply just as well to other temperate primitively eusocial taxa. However, the evidence supporting the hypothesis was only correlative: late-maturing B1 females were more likely to enter hibernation and become next year's new queens, and this was associated with there being a more male-biased sex-ratio in the mating pool when they matured. Our model assumed that caste is indeed determined by mating status, as postulated by Yanega (1989). We could then test whether the temporal pattern in the OSR that Yanega observed empirically is consistent with the caste determination mechanism that he proposed.

The main conclusion from our model is that the evolutionarily stable OSR can either increase or decrease over time, depending on parameter values. However, with the parameters in our model, a gradual increase in the proportion of males over time is found only when early workers are more valuable than late workers. If this is not the case, then so long as males lifespan is not very short relative to the period of female emergence, they have greater reproductive success when produced early, leading to a more male-biased OSR in the first time step relative to the last (Fig. 3).

Even when early-produced workers were more valuable than late-produced ones, the temporal trend in the OSR observed by Yanega (1989) appeared only under some combinations of parameter values (Fig. 4). The pattern was more likely, and more exaggerated, if there was high male mortality (so that early males were relatively less valuable), low worker mortality (m_w) and if workers were more valuable in general because they had greater

productivity (high a). Situations where workers are particularly valuable will be situations in which they can help to produce many B2 offspring, such as when resources are particularly abundant after B1 workers reach adulthood. Another such situation will be when the B2 rearing season is long (Field et al., 2010; Soucy and Danforth, 2002). However, under the MLH, a longer period during which workers contribute to producing B2, while leading to more worker production overall (unpublished results from our model), actually tends to make it less likely that the pattern in the OSR reported by Yanega (1989) occurs. This is because it reduces the benefit of producing early workers instead of late ones. The amount of time available to rear each discrete brood is likely to increase at lower latitudes or altitudes where there is a longer growing season, until the season is long enough that an extra brood can be fitted in. Under some circumstances, notably when workers are very valuable compared to hibernators in our model, selection led to a situation in which the first brood of offspring was composed entirely of females which, in the absence of males, must all become workers under Yanega's hypothesis. Broods consisting of $\approx 100\%$ workers do indeed seem to occur in some primitively eusocial taxa (e.g. Strohm and Bordon-Hauser, 2003; Packer and Knerer, 1985; Yanega, 1993). Note that conditions reducing the fitness of all hibernators, for example when founding a new nest is risky, will not alter the relative fitness of B1 hibernators and workers, since the latter contribute to reproduction by themselves helping to produce hibernators.

We note that our model considers the sex-ratio strategy of only the foundress when the MLH hypothesis is true. It does not consider the stability of such a strategy from the point of view of workers. Yanega's (1989) hypothesis implies that B1 females do not choose strategically whether to become workers or hibernators – it is simply a question of whether they happen to mate early in life, which itself depends on the OSR. The question arises as to why individual B1 females would respond to mating as a cue. In order for the MLH to be evolutionarily stable, it may also be necessary that the outcome coincides sufficiently with the reproductive interests of the female whose caste is being determined. For example, in situations where B1 females would have much greater inclusive fitness as workers than as hibernators, we would expect them to evolve to become workers regardless of their mating status. An alternative explanation for Yanega's (1989) observations is that earlier-emerging B1 females are more likely to become workers for strategic reasons, for example because early in the maturation period there is more time left in the nesting season in which to be productive as a worker (Field et al., 2010; Soucy and Danforth, 2002). Selection might then favour late production of males by foundresses, because males transmit their genes primarily by mating with females that will enter hibernation and become reproductives (except to the extent that workers occasionally lay female eggs or become replacement queens (Field et al., 2010; Yanega, 1989)). In this scenario, the direction of causation is reversed: late-maturing B1 females are more likely to choose to become reproductives, independent of their mating status, thus selecting for a later production of males and therefore a gradually more male-biased OSR in the mating pool. Reproductives might also be more likely than workers to accept mating attempts by males – since females do not need to mate in order to perform the worker role – explaining why almost all hibernating reproductives have mated (e.g. Field et al., 2010, 1998) whereas a variable proportion of workers fail to mate (e.g. Packer and Knerer, 1985). Yanega (1997) discusses this possible reversal of the direction of causality.

Our results show that under certain conditions, the MLH can lead to a gradually more male-biased OSR in the B1 mating pool over time, and could thus operate in caste determination in the way hypothesised by Yanega (1989, 1997). To further evaluate the MLH, experimental manipulations are now required. One possibility might be to provide or deny newly emerged females access to males in the lab. Even in the field, it might be possible to systematically remove

adult males as they search for females in the nesting area, and test whether a larger proportion of B1 females then become workers. Few data are currently available to test whether there is a causal relationship between mating and subsequent reproductive strategy (Plateaux-Quenu and Packer, 1998, 1960). Using lab nests of the sweat bee *Lasioglossum albipes*, Plateaux-Quenu and Packer (1998) found that 9/13 B1 females that became workers and had known mating times had mated on the day that they first left their nests after reaching adulthood. These rapidly mating workers would seem to contradict Yanega's (1989) mate limitation hypothesis, although the latency to mating for females that subsequently entered hibernation was not available for comparison. Some studies of primitively eusocial insects suggest that gene expression during development differs between larvae destined to become workers or hibernators (Hunt et al., 2010), and that when they reach adulthood, workers have smaller fat bodies than females that will enter hibernation (Hunt et al., 2010; Plateaux-Quenu and Packer, 1998; Richards and Packer, 1994). If females then use their condition as a caste-determining cue, caste might effectively be determined or biased during immature development rather than in adulthood. However, some of these data could themselves be biased because females have been categorised as workers or hibernators according to body size (e.g. Richards and Packer, 1994), or because workers and hibernators were sampled at different times of year. Experimental manipulation suggests that mating may determine caste in the only known sweat bee with perennial colonies, *Lasioglossum marginatum* (Plateaux-Quenu, 1960). Mating in *L. marginatum* occurs within the nest, but until their final year of life nests are kept closed late in the season when males are present so that males cannot gain access. By experimentally opening a nest early, Plateaux-Quenu (1960) caused females that would otherwise have been workers to be mated and become new queens. However, the unique nesting biology of *L. marginatum* suggests that its method of caste determination may not apply to other primitively eusocial taxa.

In summary, our model suggests that Yanega's (1989) mate limitation hypothesis could produce an evolutionarily stable operational sex-ratio that becomes more male-biased as the first brood mating period progresses, thus mimicking the pattern recorded by Yanega (1989), but only under a few restrictive conditions. An alternative explanation for this pattern, however, is that later-emerging first brood females are more likely to become reproductives for strategic reasons, thus selecting for later male production. The few empirical data available suggest that caste-determination mechanisms other than mate limitation may operate in some primitively eusocial taxa, while mating may indeed determine caste in *L. marginatum*. Further manipulative studies, especially in *H. rubicundus* itself, are now needed to test Yanega's (1989) hypothesis more thoroughly.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jtbi.2013.05.032>.

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