A Model of the Oviposition Behavior of *Copidosoma koehleri* Parasitoid Wasps

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Abstract

Copidosoma parasitoid wasps play an important role in controlling agricultural pests. This paper describes a complex discrete time model for the mating, oviposition, host and egg development of these wasps. The model was used to obtain optimal strategies for uninformed wasps and to test these strategies both in a statistical and evolutionary way. Furthermore experimental data provided by Ben Gurion University was used to obtain estimates of parameters for partly informed wasps. These parameters were then tested for different properties using the model. The results show that the strategy estimated from the experimental data is superior to the uninformed strategy.

Key words. Wasps, simulation, parasites, sex choice

1 Introduction

Copidosoma koehleri are small parasitoid wasps which lay their eggs into larvae of the potato tuber moth *Phthorimaea operculella*. We will therefore refer to these larvae as hosts. The female wasps have the special ability to choose the sex of each egg they lay, given they have been fertilized. A virgin wasp can only lay male eggs. When a wasp lays an egg into a host a number of wasps (clones) will develop inside the host while consuming and eventually killing it. The wasps commonly lay more than one egg into one host (super parasitism) which yields to competition within the host [3]. In order to give the female eggs an advantage they additionally develop a soldier larvae which protects the offspring by feeding on other eggs. Hosts that contain too many eggs will die prematurely before the wasps fully develop and no wasps will survive. Experiments indicate that there is a fraction of wasps mating inside the host, given it contains males and females [5]. *Copidosoma koehleri* wasps are successfully used as a biological control agent for the potato tuber moth in potato fields and in potato storage facilities since the moths are an agricultural pest in warm countries.

The objective of this paper is to describe a probabilistic model of the oviposition behavior and egg development of *Copidosoma koehleri* wasps. The model allows long term simulations with thousands of wasps with different strategies and monitoring of output values over multiple simulations. Therefore different set-ups (strategies and model parameters) can be analyzed for stability or dominance of specific strategies.

A central issue when modeling the behavior of the wasps is the amount of information about a host that a wasp can access using its senses. There are three levels of information a wasp can access. An uninformed wasp has no information about eggs that have previously been laid into a host. A partly informed wasp can sense the relatedness of eggs within a host. A fully informed wasp can also sense the sex of the eggs. First experiments indicated that if wasps do not notice whether a host can still feed additional eggs, premature host death becomes very common and the wasp population will eventually be extincted. Therefore we will refer to a wasp with only that single bit of information as an uninformed wasp. Unfortunately, the question, what information a wasp can access, can hardly be answered by practical experiments. Therefore all possible levels of information will be included into the model.

2 Assumptions

In order to make it possible to implement the model in an effective way, a number of assumptions have been made. According to observations generations do not overlap [2]. This implies that each generation starts with a number of parasitized and non-parasitized hosts. A certain fraction of wasps mate within the host so there will be a fraction of non virgin wasps directly after dispersal. Furthermore the lifespan of a female wasp is defined as the number of eggs it can lay, while the lifespan of a male is defined as the number of females it can fertilize. The **life span** parameter refers to the life span of the females and is assumed to be equal for every wasp. A wasp is assumed to be able to visit a specific number of hosts before making a choice where to lay an egg. This number is set by the **Host sample size** parameter.

3 The Model

The Model has been split into three sub models, each modeling an intuitive part (see Figure 1). The main model is the environment model which contains lists of wasps and hosts and uses both the wasp and the host sub-models for the related subprocesses.

3.1 Environment model

The purpose of the environment model is to store information about the hosts and wasps that are currently being simulated. Furthermore it models the lifespan and reproduction of hosts and the lifespan, mating and traveling of the wasps. The initial number of hosts is specified by the **Host count** parameter. Depending on the **host reproduction** parameter hosts can either die from parasites, while the survivors reproduce at a certain rate, or refresh for each generation, such that at the beginning of each generation a certain number of hosts is available. The **virility** parameters define the number of females a male wasp can fertilize on average. This takes into account that virgin's sons are generally less virile then non-virgin's. In

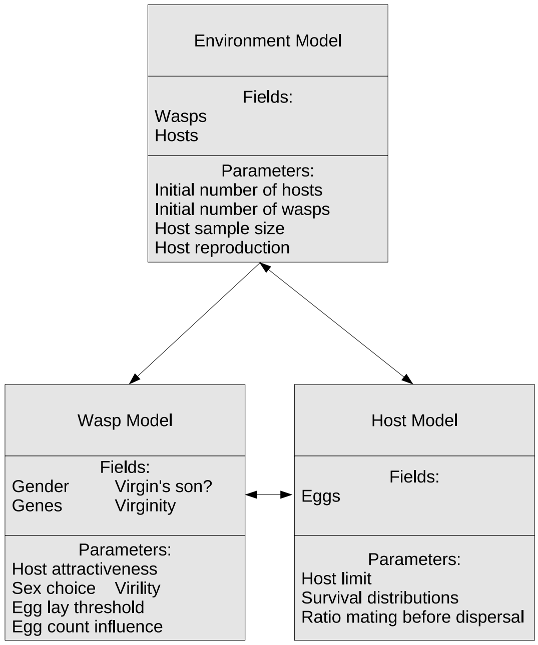

Figure 1: Model layout.

order to model the traveling of the wasps and the spatial distribution of hosts the **host sample size** parameter is used to set the number of hosts a wasp visits before deciding if and where to lay an egg.

3.2 Host model

Since hosts may die prematurely when they contain too many eggs, the host model includes both egg and host development. In order to model the possibility of premature host death, a limit for the number of wasps that can disperse out of a host has to be defined as the parameter **host limit**. For sufficient modeling of the competition between eggs inside the host, a number of parameters, the **survival distributions** are used. For the default values for these parameters data from field experiments was used [3]. These distributions define the number of dispersing wasps for each egg and for each possible configuration of eggs in a host as a matrix of normal distributions (see Figure 2). The parameter **ratio mating before dispersal** defines the ratio of females that disperse as non virgin, on condition that the host contained male eggs as well.

3.3 Wasp model

To model the host choice of the wasps, each type of host (hosts differ by the eggs they carry) is given an **host attractiveness** value. Furthermore

Brood type	Female	Male
Unrel. fem.	$\mu = 30$ $\sigma = 10$	
Rel. fem.	$\mu = 45.8$ $\sigma = 10.9$	
Unrel. male		$\mu = 24$ $\sigma = 10$
Rel. male		$\mu = 32.7$ $\sigma = 10.99$
Unrel. mix.	$\mu = 36$ $\sigma = 10$	$\mu = 10$ $\sigma = 1$
Rel. mix.	$\mu = 45.8$ $\sigma = 10.9$	$\mu = 32.7$ $\sigma = 10.99$

Figure 2: Example of a survival table. Each cell contains parameters of a normal distribution for the given case.

the parameter **egg count influence** determines how much attractiveness a host loses by carrying eggs. As a model for the oviposition behavior a matrix of Bernoulli distributions, the **oviposition matrix** (see Figure 3), is used.

	Sex of eggs		
Relation	Male	Female	Mixed
Empty	0.2		
Related	0.4	0.4	0.4
Unrelated	0.5	0.5	0.5
Mixed	0.3	0.3	0.3

Figure 3: Example of an oviposition matrix. Each cell contains the probability for laying a male egg into the given host type.

3.4 Parameters

There are two types of parameters in the model. The main parameters mainly affect the environment model and these are equal for all wasps in the simulation. The strategic parameters on the other hand, correspond to properties of the wasps and these can be different for every wasp. The model can be initialized with specific numbers of wasps following different strategies.

3.5 Implementation

To be able to implement the model to be executed by a computer, all processes have to be described mathematically. The probability of a wasp losing its virginity within a time step is calculated using the formula:

$$p_f = \frac{\sum_{k=1}^{n} u_k}{lv},$$

where n is the number of males in the population, u_k is the **virility** of wasp k, l is the **life span** of individual wasps and v is the number of virgins in the population.

In order to create a probability distribution for a sample of hosts the attractiveness value for each host is calculated and the result is normalized to a sum of 1.

$$a(h,w) = \frac{A(T_h, v_w)}{1+ie_h},$$

where $a(h,w)$ is the attractiveness of host h to wasp w, $A(T_h, v_w)$ is the **host attractiveness** of host type T_h given that the virginity of wasp w is v_w, i is the **egg count influence** parameter and e_h is the number of eggs in host h.

3.6 Statistical counters

The model contains a series of statistical counters to record specific values during a simulation. Each value is recorded for the entire population and for each of the used strategies. To follow the development of the wasp population, the population size and the number of male and female wasps are recorded. The counters for male, female and mixed broods record information about which brood types appear in the simulation. A brood is a number of wasps emerging from one host. Furthermore the number of male and female eggs are recorded. Environmental values are the number of empty hosts, the size of the host population and the number of premature host deaths.

4 Optimal strategies for uninformed wasps

A large number of experiments has been conducted using the simulation. The aim of these experiments was to determine optimal values for the parameters of an uninformed wasp. An uninformed wasp is unable to tell the sex and relatedness of eggs that were previously laid into a host. Experiments showed that wasps that cannot tell whether a host can feed any more eggs, are unable to survive. This is due to the fact that hosts containing too many eggs die prematurely. Therefore we assume hosts to get more unattractive with a higher number of eggs inside.

The host attractiveness and **sex choice** parameters of the model have been set to equal values for each host type, so that the wasps are uninformed. The population is initialized with 1000 female wasps with a virgin ratio of 5 percent. Experiments indicated that the number of hosts scales the size of the population linearly.

Since these starting conditions do not match a realistic wasp population an initial number of generations is discarded until the simulations reaches a steady state, where the output is independent of the starting conditions. Experiments showed that discarding a constant number of 20 generations is usually sufficient. In order to obtain a large number of samples, 200 generations have been simulated for each case.

An important measure for the performance of a strategy is the stability of the population over a large number of generations. The variance of the numbers of male and female wasps and of the population size are used to quantify this. Simulations in marginal cases showed partly stability where the population is stable at some time intervals and unstable in others (see Figure 4).

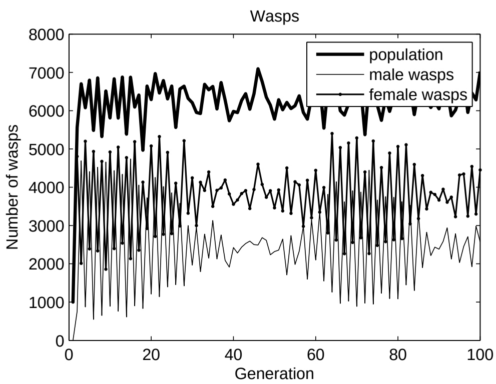

Figure 4: Plot of partly stable output.

4.1 Sex choice

Since wasps do not have information about the types of eggs in the host, the **sex choice** parameter comes down to a single value between 0 and 1. Where 1 means only laying male eggs. The range of this parameter has been covered with 100 replications of the model. The results indicate a clear disadvantage of a strong preference for laying female eggs. This can be explained by virginity of the wasps. A generation of wasps that (nearly) always lays female eggs will have a lack of males in the following generation. Therefore the new female wasps will not (entirely) get fertilized and therefore be unable to follow their strategy to lay preferably female eggs. This will cause high instability since every second generation will have a lot of males, while the other generations have a lot of females. The results of the simulations are illustrated in Figure 6. The local minimum at 0.33 can be explained as an artifact caused by partly stability.

4.2 Superparasitism

The **egg lay threshold** parameter determines the amount of superparasitism in the simulation. Figure 5 shows the influence of the parameter on the number of eggs a host receives on average. Experiments have been conducted to obtain information about the influence of the parameter on the population. The experiments showed that the parameter mainly influences the size of the population and has only little influence on stability. The output of the simulations is illustrated in Figure 7 where a maximum in population at a **egg lay threshold** value of 0.3 occurs. This indicates that it is best to lay 2 eggs into each host.

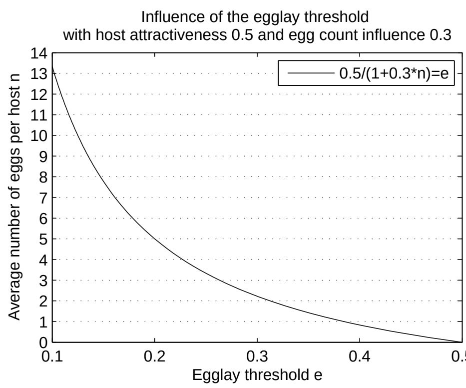

Figure 5: Influence of **egg lay threshold** parameter on superparsitism.

4.3 Life span

Since a wasp population quickly outnumbers the host population a **life span** of just 1 egg seems to be sufficient to utilize all hosts. A number of experiments with different **life spans** and stable values for the other parameters confirmed this hypothesis. On the other hand when using unstable values for the **sex choice** parameter the experiments showed that it is possible to stabilize these strategies by increasing the **life span** of the wasps. This is illustrated in Figure 8. The local minimum in the variances at a life span of 22 can be explained as an artifact due to partly stability.

4.4 Optimality testing

An optimal strategy should maximize the population size and minimize the variance. Since only a little ratio of male wasps is required to fertilize the females, another objective is to maximize the number of females. According to the performed experiments, a **sex choice** of 0.6, an **egg lay threshold** of 0.3 and a **life span** of 20 eggs seem to be optimal. To test this hypothesis this strategy was compared pairwise to a number of other strategies using a paired-t test [1]. In order to obtain data 100 simulations with each 100 generations for 100 hosts have been performed for each strategy. The first 20 generations are again discarded. Each strategy is then compared to the strategy defined above by means of stability, number of females and population size. As a measure of stability, the sum of the variances of the population and of the male and the female wasps, is used. The higher the variance the more instable the strategy. Since the variance is dependent on the population size we scale it by dividing by the population size. The data of the hypothesis strategy is then compared to the output of each of the other strategies by creating a paired-t confidence interval for the difference of the strategies and see with what confidence the hypothesis strategy is better.

Table 1 shows that the hypothesis strategy is more stable than the other strategies and mostly produces more females and larger populations. The strategies with a higher **sex choice** value, so with a stronger preference for male eggs, result in larger populations but, as to be expected, in lower numbers of females.

A second approach is to simulate a population of the hypothesis strategy and try to invade it with a small number of wasps following a different strategy. If a strategy cannot be invaded by other strategies, the strategy is referred to as evolutionary stable [4].
In order to test these properties, a simulation is initialized with a population of 1000 wasps of one strategy and 10 invading wasps following a different strategy. For each pair of strategies 20 simulations of 100 generations with 100 hosts have been performed.

Table 2 shows the results of these experiments.

s	e	l	Mean difference	φ
0.2	0.3	20	$\bar{p} = 867.03$	> 0.99
			$\bar{f} = -431.49$	< 0.01
			$\bar{v} = -10.68$	> 0.99
0.5	0.2	25	$\bar{p} = 4232.4$	> 0.99
			$\bar{f} = 1647.8$	> 0.99
			$\bar{v} = -19.74$	> 0.99
0.5	0.3	10	$\bar{p} = 159.23$	> 0.99
			$\bar{f} = -652.89$	< 0.01
			$\bar{v} = -4.31$	> 0.99
0.5	0.3	20	$\bar{p} = 54.20$	> 0.99
			$\bar{f} = -314.09$	< 0.01
			$\bar{v} = -0.19$	> 0.99
0.5	0.3	25	$\bar{p} = 918.48$	> 0.99
			$\bar{f} = 326.95$	> 0.99
			$\bar{v} = -1.52$	> 0.99
0.5	0.4	25	$\bar{p} = 2063.2$	> 0.99
			$\bar{f} = 1066.4$	> 0.99
			$\bar{v} = -11.31$	> 0.99
0.7	0.3	20	$\bar{p} = -64.20$	< 0.01
			$\bar{f} = 366.40$	> 0.99
			$\bar{v} = 0.16$	0.2
0.9	0.3	20	$\bar{p} = -254.94$	< 0.01
			$\bar{f} = 1506.6$	> 0.99
			$\bar{v} = 2.91$	< 0.01

*In this table s is the **sex choice**, e is the **egg lay threshold**, l is the **life span**, $\bar{p}$ is the mean difference in population size, $\bar{f}$ is the normalized mean difference in in number of females, $\bar{v}$ is the normalized mean difference in variance and φ is the confidence for the hypothesis strategy being better than the tested one by means of higher population or lower variance.*

Table 1: Optimality of hypothesis strategy.

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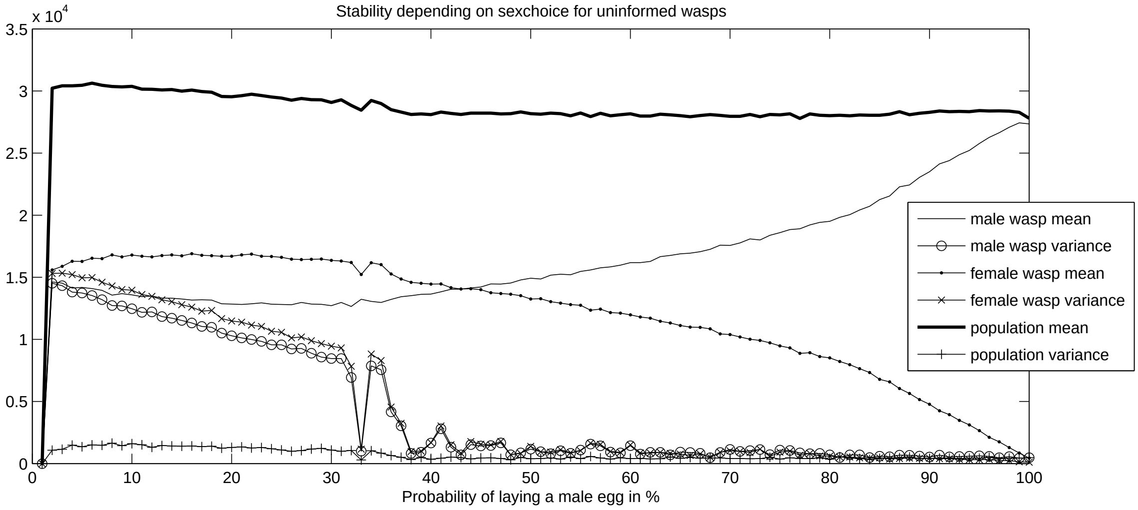

Figure 6: Sexchoice output.

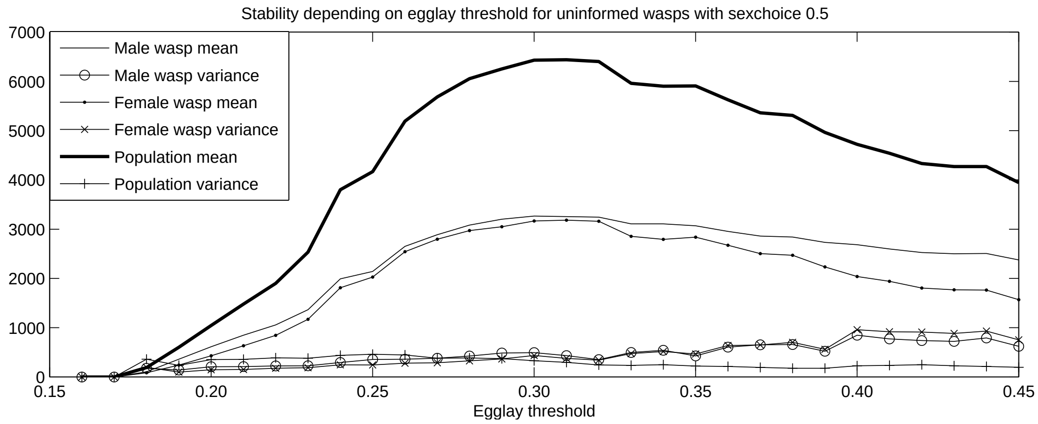

Figure 7: Egg lay threshold output.

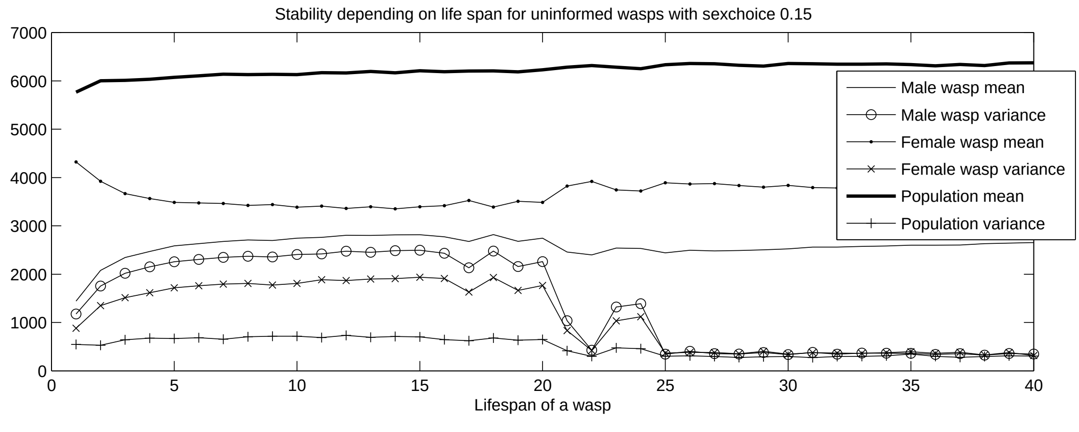

Figure 8: Life span output.

s_p	e_p	s_i	e_i	n_i
0.1	0.3	0.6	0.3	0
0.1	0.3	0.2	0.3	0
0.2	0.3	0.1	0.3	6
0.5	0.3	0.1	0.3	15
0.6	0.3	0.1	0.3	14
0.6	0.3	0.8	0.3	0
0.6	0.3	0.6	0.25	20
0.6	0.3	0.6	0.35	0
0.6	0.35	0.6	0.3	0
0.7	0.3	0.6	0.3	8
0.8	0.3	0.6	0.3	10

In this table s_p is the ***sex choice*** *of the main population, e_p the* ***egg lay threshold*** *of the main population and s_i, e_i are the same parameters for the invading wasps. n_i is the number of times the invasion was successful within the 20 performed simulations.*

Table 2: Successful invasions of strategies.

It shows that although the hypothesis strategy is optimal from a statistical perspective it does not seem to be evolutionary stable since it can be invaded by a number of strategies either laying more eggs or having a stronger preference for females.

5 Optimal strategies for partly informed wasps

A wasp that can sense eggs inside a host and determine its relation to the eggs is referred to as a partly informed wasp. In order to describe a **sex choice** strategy for a partly informed wasp four values between 0 and 1 are required. Each value stands for the average **sex choice** for one of the four host types. Furthermore four **host attractiveness** values for each of the host types are required.

At Ben Gurion University a number of experiments with real wasps have been conducted in order to gain information about the **sex choice** behavior. Wasps have been put together with hosts and were allowed to lay a controlled number of eggs. After the eggs developed, it was checked whether it was an all female, an all male or a mixed brood.

The results of these experiments are shown in Table 3. The first row, vms, refers to an experiment where a virgin wasp lays one egg into one host, is then mated and lays another egg into the same host. The second row, vmd, shows the brood sizes that resulted from a virgin wasp laying an egg into one host and another mated wasp laying another egg into the same host. The third row, mms, gives the results for a mated wasp laying two eggs into one host and the last row, mmd, shows the results of two different mated wasps laying two eggs into one host.

	male	female	mixed
vms	10	4	2
vmd	5	1	6
mms	4	7	2
mmd	3	7	3

Table 3: Brood sizes that resulted from experiments with real wasps.

	male	female
vms	26	6
vmd	16	7
mms	10	16
mmd	9	17

Table 4: Estimated number of eggs within the hosts from experiments with real wasps.

Table 4 shows estimations of the number of eggs in the real experiments shown in Table 3. Since we cannot estimate **host attractiveness** values from these experiments we focus on the four **sex choice** parameters. From the results ranges for each of the four **sex choice** values were estimated. In case of an empty host the wasps seem to prefer laying female eggs since the number of female eggs is higher for the last two experiments. Since in case of the second experiment more females were produced than in the first case there seems to be a preference for females given a non self parasitized host. For self parasitized hosts there seems to be a preference for males. Unfortunately we cannot make any assumptions about the mixed parasitized host type, since no more than 2 eggs were laid into one host in the experiments.
In order to estimate the benefit of using the extra information, a number of strategies has been created that match the experimental data. These strategies are listed in Table 5.

These strategies were now statistically tested against the optimal uninformed **sex choice** strategy of laying a male egg in 60% of the cases. For each of the strategies 100 simulations with 100 gener-

S	s_e	s_s	s_n	s_m
h_1	0.2	0.66	0.33	0.5
h_2	0.2	0.66	0.2	0.5
h_3	0.0	1.0	0.0	0.0
h_4	0.0	1.0	0.0	0.5

*In this table s_e is the **sex choice** given an empty host, s_s given a self parasitized host, s_n, given a non self parasitized host and s_m is the **sex choice** given a mixed parasitized host (contains both related and unrelated eggs).*

Table 5: Hypothesis strategies for partly informed wasps.

ations with each 100 hosts have been performed similar as in Section 4.4. The results of the tests are shown in Table 6.

The $\bar{f}$ values show that each of the hypothesized partly informed strategies produces significantly higher numbers of females. At the same time the populations are smaller and the variance is slightly increased. Since the variances are still low, all four strategies can be viewed as superior to the uninformed strategies. The highest number of females was observed at the third strategy h_3.

In order to check for evolutionary stability of the strategies, more experiments have been performed to test whether the partly informed strategies can invade or can be invaded by the uninformed strategy. These experiments clearly show that none of the hypothesized partly informed strategies can be invaded by the uninformed strategy. Also all of the partly informed strategies manage to invade an uninformed wasp population. These results are listed in Table 7.

s_e	s_s	s_n	s_m	Mean diff.	φ
0.2	0.66	0.33	0.5	$\bar{p} = -138.12$ $\bar{f} = 854.55$ $\bar{v} = 0.81$	< 0.01 > 0.99 < 0.01
0.2	0.66	0.2	0.5	$\bar{p} = -190.26$ $\bar{f} = 1032.6$ $\bar{v} = 1.13$	< 0.01 > 0.99 < 0.01
0.0	1.0	0.0	0.0	$\bar{p} = -264.56$ $\bar{f} = 1373.2$ $\bar{v} = 2.24$	< 0.01 > 0.99 < 0.01
0.0	1.0	0.0	0.5	$\bar{p} = -268.73$ $\bar{f} = 1367.6$ $\bar{v} = 2.19$	< 0.01 > 0.99 < 0.01

*In this table s_e is the **sex choice** given an empty host, s_s given a self parasitized host, s_n, given a non self parasitized host and s_m is the **sex choice** given a mixed parasitized host. $\bar{p}$,$\bar{f}$ and $\bar{v}$ are the mean differences for population, females and variance respectively. φ is the probability of the strategy being better than the optimal uniformed strategy in the respective category.*

Table 6: Test of the hypothesized partly informed strategies against the optimal uninformed strategy.

S_p	S_i	n_i
S_u	h_1	9
S_u	h_2	8
S_u	h_3	9
S_u	h_4	11
h_1	S_u	0
h_2	S_u	0
h_3	S_u	0
h_4	S_u	0

In this table S_p is the strategy of the population and S_i is the strategy of the invaders. n_i is the number of successful invasions. S_u is the uniformed strategy.

Table 7: Successful invasions of partly and uninformed wasps.

6 Concluding remarks

This paper describes a discrete time model for the oviposition behavior of *Copidosoma koehleri* parasitoid wasps. The possible levels of information are included into the model. Furthermore we showed that the model can be used to find and test strategies for different properties. We performed a large number of simulations to determine optimal strategies for uninformed wasps. Then we performed statistical tests to confirm our hypothesis. Furthermore we performed simulations with wasps of different strategies and tested for evolutionary stability.

Data from experiments with real wasps has been provided by University of Haifa. We used this data to obtain estimates for the sex choice parameters for partly informed wasps. Both statistical and evolutionary stability experiments showed that these estimates are superior to the uninformed strategy.
In order to obtain more detailed information about optimal strategies and evolutionary stability, larger numbers of simulations should be performed. For example 2^k-factorial design [1] can be used to obtain better approximations for the parameters of the model. Due to the high number of simulations required to do so, this was not possible within the time frame of this paper.

We thank Dr. Tamar Keasar from University of Haifa for providing accurate information and helpful discussion.

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Appendix A: Parameters

Main parameters

Generations	Number of generations to simulate.
Lifespan	Lifespan of a wasp in number of eggs it can lay.
Host count	Number of hosts at initialization.
Host limit	Number of wasps one host can feed on average.
Host refresh	Toggles whether hosts refresh for each generation.
Host-reproduction	Rate of reproduction of surviving hosts, if hosts do not refresh.

Strategic parameters

Wasp count	Number of wasps at initialization.
Sex ration	Ratio of males at initialization.
Virgin ratio	Ratio of virgins at initialization.
Egg count influence	Influence of number of eggs on host attractiveness.
Egg lay threshold	Attractiveness Threshold for laying eggs.
Virgin's son virility	Virility of a son of a virgin.
Non virgin's son virility	Virility of a son of a non virgin.
Host sample size	Number of hosts a wasp visits before laying an egg.
Ratio mating before dispersal	Ratio of wasps mating within the host.
Virgin host attractiveness	Attractiveness for each host type for virgins.
Non virgin host attractiveness	Attractiveness for each host type for non virgins.
Oviposition matrix	Probability of laying a male egg for each host type (see Figure 6).
Survival matrix	Mean and standard deviation for each gender for each brood type (see Figure 2).

Appendix B: Flowcharts

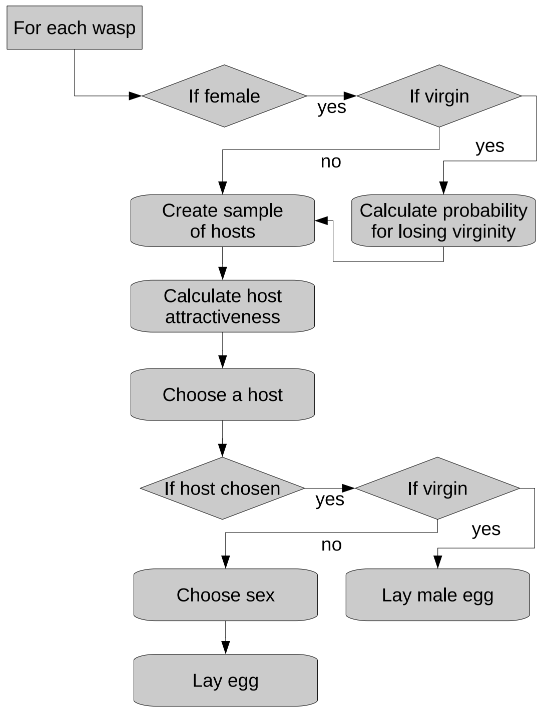

Figure 9: Wasp flowchart.

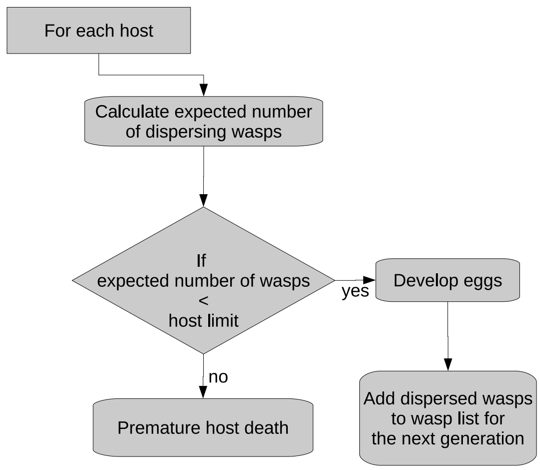

Figure 10: Host flowchart.

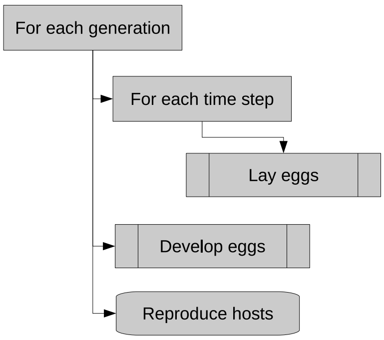

Figure 11: Environment flowchart. ”Lay eggs” corresponds to Figure 9, ”Develop eggs” corresponds to Figure 10