Title:

A novel two-generation approach to the population dynamics of gregarious parasitoids

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Abstract

Parasitoids as one of the important natural enemies maintain balance of natural ecosystems. Their population density is traditionally measured as the number of individuals and from number of females and maternal fertility is generally predict parasitoids population dynamics. Here, we propose the novel two-generation approach, which includes typically relationship of gregarious parasitoids (the clutch size – body size – fertility correlation) and which provides more accurate result for determining the individual fertility levels of parasitoids. Using this novel approach, on the model species *Anaphes flavipes*, we show that the same fertility in the F1 generation can cause distinctly different fertility values in the F2 generation, even with the same number of hosts, lower fertility in the F1 generation can cause higher fertility in the F2 generation. Based on our results, we propose determination of parasitoids fertility from body size and use a two-generation approach for gregarious parasitoids that have a major impact on biodiversity. In addition, we showed that by combination knowledge of ecosystem characteristics that change the parasitoids clutch size and a two-generation approach, future parasitoids population dynamic and ecosystem stability can be relatively easily predicted.

Main Text

Parasitoids are one of the important natural enemies that play an important role in the biodiversity and maintain balance of natural ecosystems[1,2]. Using knowledge of parasitoids species and their population dynamics, we can predict the stability of the ecosystem. Population dynamics not only for parasitoids is traditionally determined from number of individuals and then from maternal fertility[3,4]. Here we propose that this approach is suitable for determination the population dynamic of solitary parasitoid, which lay one or more egg into a single host, but their larvae are predatory and only one survives[5]. If only one offspring completes larval development in a host, its body size and their future fertility are directly proportional[e.g. 6], by which the population dynamics of solitary parasitoids can be predicted (Fig. 1a,b). In contrast, the gregarious parasitoids with tolerant larvae employ a strategy in which one or more parasitoids may develop in a single host[5]. The mothers can deliberately manipulate with offspring fertility, because they decide on the offspring body size and their future fertility by choosing clutch size[e.g.7,8,9]. In this study, on a model of gregarious parasitoid *Anaphes flavipes*, for the first time we determined the future offspring fertility from their body size (Table 1) and we experimentally verified, that the population dynamics of gregarious parasitoids cannot be predicted using the traditional approach (maternal fertility),

because the same fertility in the F1 generation can cause very different fertility in the F2 generation (Fig. 1b,c; Suppl. Mat. 1).

Table 1. The fertility of the gregarious parasitoid *Anaphes flavipes* among different clutch sizes (mean ± 95% confidence interval). The column "c)" shows that the fertility of females in the F2 generation can be reduced by almost half, due to the different distribtion of 12 offspring into clutch size.

Clutch size	a) Fertility of one offspring	b) Fertility of all offspring per host	c) Fertility of 12 offspring
1	19.3 ± 1.3	19.3	232 (100%)
2	13.8 ± 0.3	27.6	166 (72%)
3	11.2 ± 0.4	33.6	134 (58%)
4	10 ± 1.5	40	120 (52%)

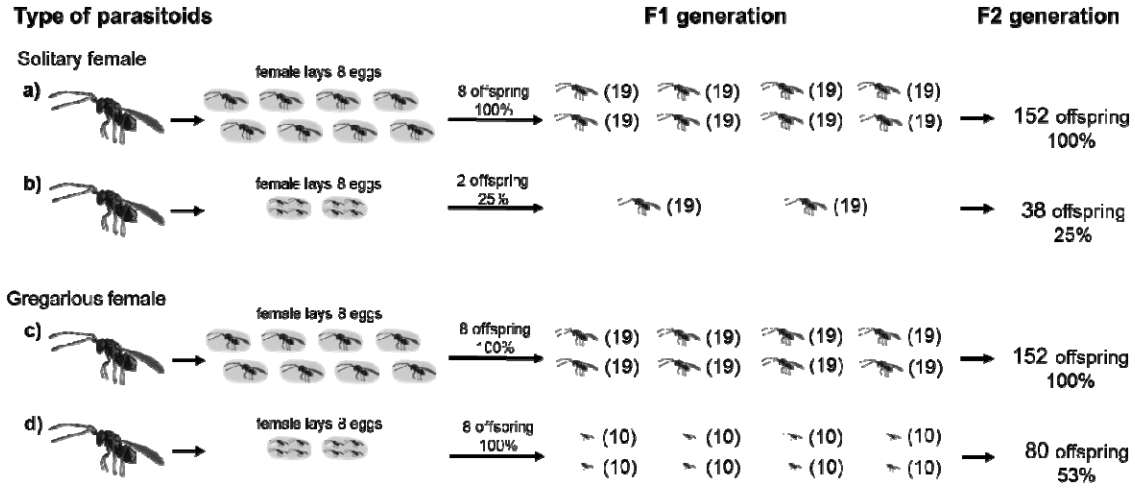

Fig. 1. The different fertilities obtained in the F2 generation for solitary and gregarious parasitoids with different numbers of host eggs (to simplify the model, the fertility values (shown in parentheses) are rounded, and male offspring are not shown; these simplifications are explained in detail in the methods section *Exp. 1*).

Here, based on our results, we propose the two-generation approach, which takes into account the clutch size – body size – fertility correlation. Using this approach, we obtain a more accurate view of the population dynamic of gregarious parasitoids compared to the traditional approach of maternal fertility.

As shown in Fig. 1, parasitoid fertility generally depends on the host population density and the number of eggs a female has available (fecundity)[10]. Here, using two generation approach, in Fig. 2a,b, we show that with the same number of hosts and the same number of offspring, females decide about offspring fertility by decision about clutch size. The study of clutch size is one of the oldest and most enduring topics in behavioural ecology[11]. It is known that some combinations of the number and sex ratio of offspring developing in one host are more advantageous than other[12]. These combinations are favoured by natural selection and over time they should stabilize to maximize individual fitness[12,13]. Using two-generation approach, we have clarified the advantage of clutch size combinations in form of higher fertility obtained in the F2 generation. Using this, the females can

deliberately manipulate their fertility in the F2 generation by choosing suitable clutch size combinations in F1 generation (Fig. 2a,b). Even, with the same number of hosts, lower fertility in the F1 generation can cause higher fertility in the F2 generation (Fig. 2b,c). Using our experiments, we have shown with particular number of hosts, wasps did not distribute their offspring randomly, but chose combinations that were described as favoured[14] and we experimentally showed that they increased total fertility of F1 generation (paired t-test, $t = 8.932$, $p < 0.001$) (Suppl. Mat. 2).

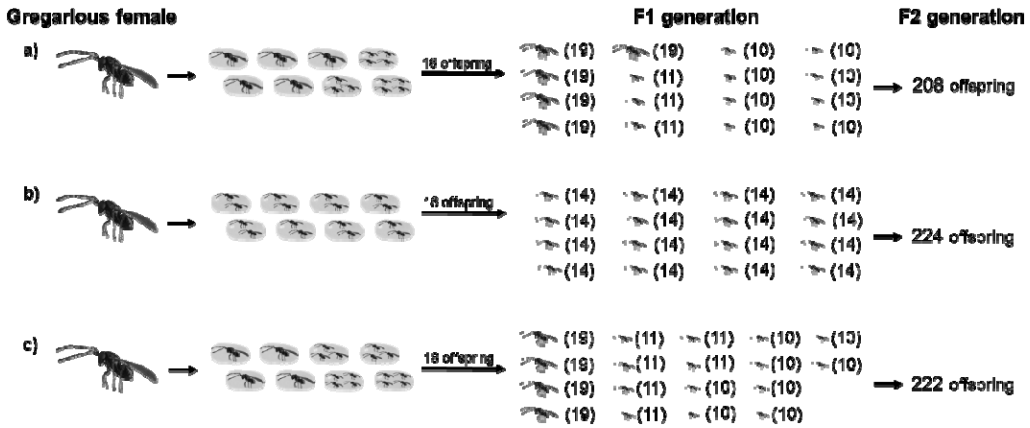

Fig. 2. The different fertility values obtained in the F2 generation of females with the same number of host eggs and the same (2a, b) or different fertility values in the F1 generation (2c) (to simplify the model, male offspring are not shown, explained in detail in the methods section *Exp. 2*).

In the second part of this study, we show the applied use of a two-generation approach for determine the population dynamics of parasitoids in the field. We are aware that determining the parasitoid fertility from clutch size requires extensive measurements difficult to apply in the field. As an alternative, we propose obtaining knowledge of factors such as host availability[15,16], host characteristic[17] or the presence of a predator of the host[15] with which the parasitoid female "*consciously*" changes the clutch size and, thus, the body sizes of the offspring and their future fertility values. With knowledge of these factors in a specific environment, we can expect a decrease or increase in parasitoid clutch size and fertility and determine their population dynamics using a two-generation approach. As an example, we know that with the presence of a predator of host eggs, *A. flavipes* females lay more offspring into the host[15], probably because some predators prefer non-parasitized hosts[18], but at the same time the parasitoids female do not reduce fertility in F1 generation. The traditional approach of maternal fertility will not show changes in fertility. However, due to parasitoids higher clutch size, their offspring will be small with low fertility in F2 generation[7], so a low population density of parasitoids can be expected in localities with predator of host.

Interestingly, here, we confirm that clutch size can also be affected "*unconsciously*" by the presence of non-native hosts. In our experiment, the clutch sizes of *A. flavipes* females with 12 host eggs available for parasitisation were lower than those of females with 6 available host eggs due to the distribution of offspring among multiple hosts (LMM, $\chi^2 = 6.824$, $p = 0.009$; Fig. 3a, b). Similarly, females with 6 native hosts and 6 non-native hosts in which the offspring were unable to complete larval development (Fig. 3c) had lower clutch sizes than females with 6 native hosts (LMM, $\chi^2 = 12.193$, $p < 0.001$; Fig. 3b), and no significant difference in clutch size was observed compared to

females with 12 native hosts (LMM, χ^2 = 0.709, p = 0.4; Fig. 3a). The presence of a non-native host "*unconsciously*" reduces the fertility of females in the F1 generation; at the same time, due to the distribution of offspring among multiple hosts, the presence of a non-native host reduces the clutch size and increases the fertility of offspring in the F2 generation (Fig. 3c). Using the traditional one-generation approach, we will expect reduced fertility by almost half in locations with a non-native host. However, from a two-generation approach, we will expect reduced fertility on these locality, but only partially, because females by offspring distribution both the non-native and native host, reduce their clutch size in the native host and thus increase the individual offspring fertility. If we use, a two-generation approach with measuring of certain clutch size will give us more accurate results; in these circumstances, the fertility of individuals in the F1 generation in the presence of a non-native host decreases by 50% (Fig. 3b, c), but among individuals in the F2 generation, fertility decreases by only 27% (Fig. 3b, c).

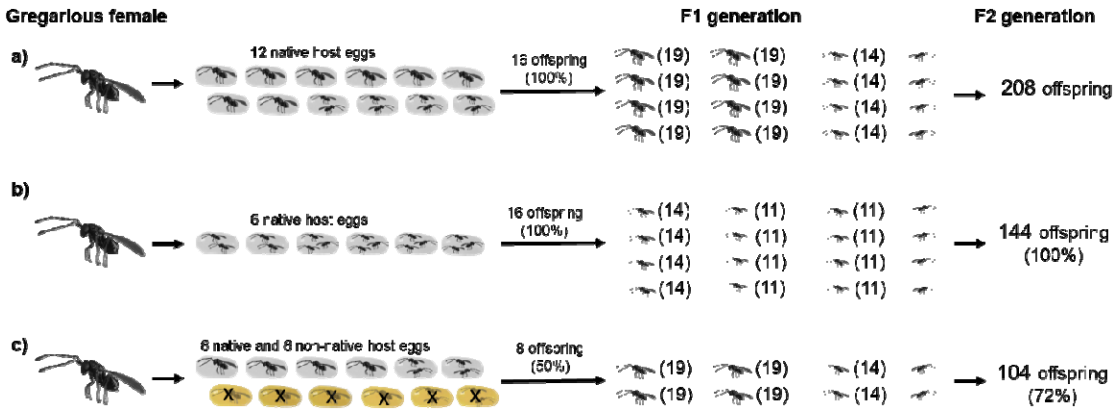

Fig. 3. The different fertility values obtained for the F2 generations of three groups of females: a) females with 12 available native host eggs; b) females with 6 available native host eggs; and c) females with 6 available native host eggs and 6 available non-native host eggs (the offspring sex ratio of *A. flavipes* is 3:1 (male:female)[14]; females are shown as (left oriented) and males as (right oriented); explained in detail in the methods section *Exp. 3*).

CONCLUSION

Based on the results of this study, we propose for parasitoid species that have a major impact on ecosystem stability, determine their fertility from body size. Thanks to, we can assume favoured advantageous clutch size combinations and a two-generation approach can be used to predict their population dynamics, which will provide more accurate results than the traditional approach[3,4]. In addition, we have shown that the population dynamics of parasitoids can be predicted by factors based on which parasitoids females *consciously* or *unconsciously* change clutch size and thus determine the offspring fertility in the F2 generation.

Materials and Methods

Parasitoids

Anaphes flavipes were reared from host eggs (*Oulema* spp.) collected in cereal fields in Prague (50.136°N, 14.363°E) from the end of April until the end of June in 2012, 2019 and 2020. The

parasitised host eggs were stored in Petri dishes with moistened filter papers until adult wasps emerged. These "wild" wasps were used as an initial population from which the next generations of parasitoids were reared in an environmental chamber at 22±2°C with 40–60% relative humidity and continuous illumination. Subsequent generations of females and males were used for experiments. Mated females (not older than 24 hours post-emergence) were placed in Petri dishes with host eggs. The females were not fed before the start of the experiment or during the experiment, and they had free access to water.

Host species

The non-native host species *Gastrophysa viridula* (DeGeer, 1775) and native hosts of the *Oulema* species complex (including two very ecologically close species, *O. duftschmidi* (Redtenbacher, 1874) and *O. melanopus* (Linnaeus, 1758)) were used; these species were used identically as in our previous studies[e.g.7,15] and in other studies[e.g.14] because they are determined only on the basis of genital preparation[19]. In the current study, the host culture was established from adults collected in Prague (50.136°N, 14.363°E) and in Police n/Met (GPS: 50.527°N, 16.245°E). The adults were kept in plastic boxes with moistened filter papers, were fed, and had unlimited access to water. They were allowed to lay their eggs on leaves at 22±2°C, a relative humidity of 40–60% and a 16:8-h L:D cycle. We used host eggs no older than 24 hours.

Laboratory experiments

All laboratory experiments were performed in Petri dishes (8.5 cm) in a thermal cabinet at 22±2°C and 40–60% relative humidity under a 16:8-h (L:D) photoperiod. Individual parasitized host eggs were moved into 1.5-ml plastic tubes on the 9th or 10th day after parasitisation and stored at the same temperature in a thermal cabinet. The number and sex ratio of wasps that emerged from each parasitised host egg were measured. The body size of the parasitoids was measured using temporary microslides according to established methodology[7].

Exp. 1) Determining fertility in the F2 generation

Each female (n=81) had 12 host eggs available for parasitisation for 8 hours. We measured the body sizes of the females and their fertility values to determine the relationship between body size and fertility. After this, we measured the clutch size and body sizes of the offspring (n=441) to determine fertility from the body size of *A. flavipes*. Data from these experiments were used for studies[7,13] focused on the body size, fertility and factors influencing the clutch size of *A. flavipes* female parasitoids (Suppl. Mat. 1).

Exp. 2) Determining advantageous combinations of clutch sizes

Each female (n=19) had 12 host eggs available for parasitisation for 8 hours. We determined the fertility values of the offspring in the F2 generation of each female. While maintaining the number of parasitized host eggs and the number of offspring (without male fertility), we determined the most

advantageous (highest fertility values in the F2 generation) and least advantageous (lowest fertility values in the F2 generation) combinations for each female and compared them with reality (Suppl. Mat. 2).

Exp. 3) Effect of non-native hosts on clutch size

Each female had host eggs available for parasitisation for 8 hours in three groups: 1) 12 native hosts (n=19); 2) 6 native hosts (n=11); and 3) 6 non-native and 6 native hosts (n=15) (Suppl. Mat. 3).

Statistical data processing

We estimated the effect of the F0 female reproductive strategy on F1-generation fertility in a two-step analysis. First, we modelled linear regression between *A. flavipes* female wing length and fertility (both with normal distributions) based on measurements of wasps used in a previous study[5]. The parameters of the regression curve were calculated in Statistica 7 (StatSoft, Inc., 2004).

In the second step, we calculated the hypothetical fertility of each female wasp used in the current study by applying regression parameters to their known wing lengths. The total wasp fertility per host was calculated by multiplying the sum of the hypothetical fertilities of the female offspring of each host by the mean rate of female offspring for each level of the total number of siblings per host (levels: 1, 2, 3, 4). Since the ultimate aim of this model was to estimate the population dynamics of the wasps, male fertility was considered to be zero.

To test the hypothesis that F0 females do not lay eggs into hosts randomly but instead select egg distributions that provide higher total fertility to the F1 generation, we compared the median F1 fertility of all possible combinations for each female with the one actually selected by the female by means of a paired t-test. The test was calculated in Statistica 7 (Statsoft, Inc., 2004).

The confidence intervals were calculated in R 4.0.1[20].

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