Title:

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

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Abstract

The population density of animals is generally measured as the number of individuals. Here, by using gregarious parasitoids as models, we show that this traditional approach omits one important parameter: the clutch size – body size – fertility correlation. Using this correlation, female gregarious parasitoids deliberately adjust the number of offspring laid in a host, determining not only the number of offspring but also the body sizes and reproductive potentials of those offspring. We propose a new two-generation approach for determining the population density of gregarious parasitoids. Using this new approach, which includes the clutch size – body size – fertility correlation, we show that the same fertility in the F1 generation can cause distinctly different fertility values in the F2 generation, and that, even with the same number of hosts, lower fertility in the F1 generation can cause higher fertility in the F2 generation. Our two-generation approach provides a new perspective for determining the individual fitness levels of gregarious parasitoids with new options for the modelling of parasitoid population dynamics.

Main Text

From the ecological point of view of reproductive strategy, parasitoids, the most diverse group in the world, can be divided into two main groups: solitary and gregarious. Female solitary parasitoids generally lay one egg, or sometimes multiple eggs, into a single host, and their larvae are predatory and are more mobile than those of gregarious parasitoids. If more than one egg is oviposited, the larvae compete until only one survives[1]. The gregarious strategy has evolved numerous times (independently at least 43 times in 26 different families of Hymenoptera) from the solitary ancestral state[2]. Gregarious parasitoids with tolerant larvae employ a strategy in

which one or more parasitoids may develop in a single host[1]. Their tolerance model assumes that, while sharing the same host, tolerant gregarious larvae will always be killed by predatory solitary larvae[3]. Therefore, the gregarious strategy should provide to its holders some other advantages to compensate for this handicap.

One of the main benefits of the gregarious strategy is the efficient use of the host in the form of multiple individuals developing in a single host; this is particularly beneficial when the host is scarce. However, for many gregarious parasitoids, a higher clutch size reduces the body size of the offspring[4] and also affects the future fertility of the offspring[5] (i.e., the clutch size – body size – fertility correlation)[6]. To our knowledge, for the first time, we determined the fertility of the F1 generation of gregarious parasitoids from different clutch sizes using the body size of the offspring (Table 1).

Table 1. The fertility of the gregarious parasitoid *Anaphes flavipes* among different clutch sizes (mean ± 95% confidence interval).

Clutch size	Fertility of one offspring	Fertility for all offspring per host
1	19.3 ± 1.3	19.3
2	13.8 ± 0.3	27.6
3	11.2 ± 0.4	33.6
4	10 ± 1.5	40

We experimentally confirmed a more effective use of hosts by gregarious parasitoids with a scarcity of hosts compared to solitary parasitoids (Fig. 1b, d), and we showed that the same fertility among females in the F1 generation may provide distinctly different fertilities in the F2 generation (Fig. 1c, d; Suppl. Mat. 1).

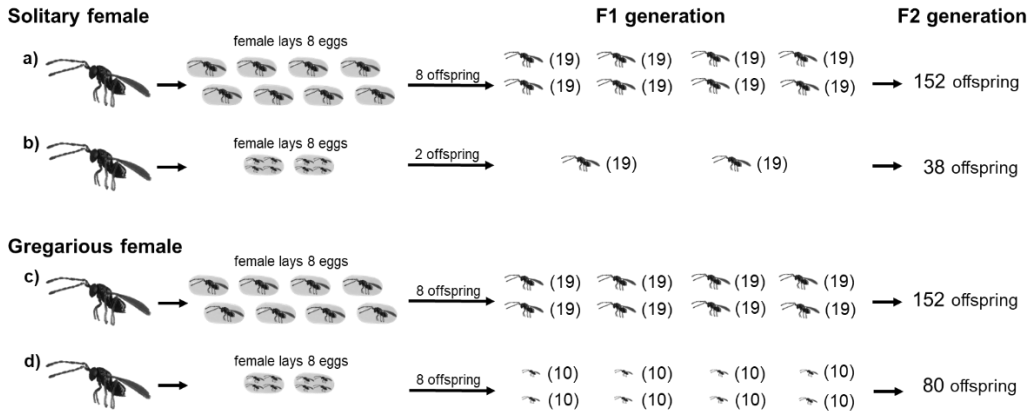

Fig. 1. The different fertilities obtained in the F2 generation for solitary and gregarious parasitoids with different numbers of host eggs (to simplify the model, the fertility values (shown in parentheses) are rounded, and male offspring are not shown; these simplifications are explained in detail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

Based on our results, we propose that by using our two-generation approach, which takes into account the clutch size – body size – fertility correlation, we obtain a more accurate view of the population densities of parasitoids compared to the traditional approach of measuring the number of individuals[7,8]. For illustration, we present a hypothetical scenario in which the same number of equally large *A. flavipes* parasitoids are released for biological control at two sites with different numbers of hosts (low and high). The population densities of the parasitoids, measured by abundance in the F1 generation (12 – 14 days after parasitoid release[9]), would be approximately the same at both sites, and differences would only be observed in the body size of the F1 offspring. However, the size of the parasitoid population in the F2 generation (24 – 28 days after parasitoid release[9]) would be notably lower in the site with low host population density due to the reduced body sizes of offspring in the F1 generation (Fig. 1c, d).

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, we show that with the same number of hosts and the same number of offspring, females of gregarious parasitoids can deliberately manipulate fertility in the F2 generation by choosing suitable clutch size combinations in F1 generation (Fig. 2a, b). Moreover, with the same number of hosts, lower fertility in the F1 generation can cause higher fertility in the F2 generation (Fig. 2b, c). When parasitizing a particular number of hosts, wasps did not distribute their offspring randomly, but chose combinations that increased total fertility of F1 generation (paired t-test, $t = 8.932$, $p < 0.001$) (Suppl. Mat. 2).

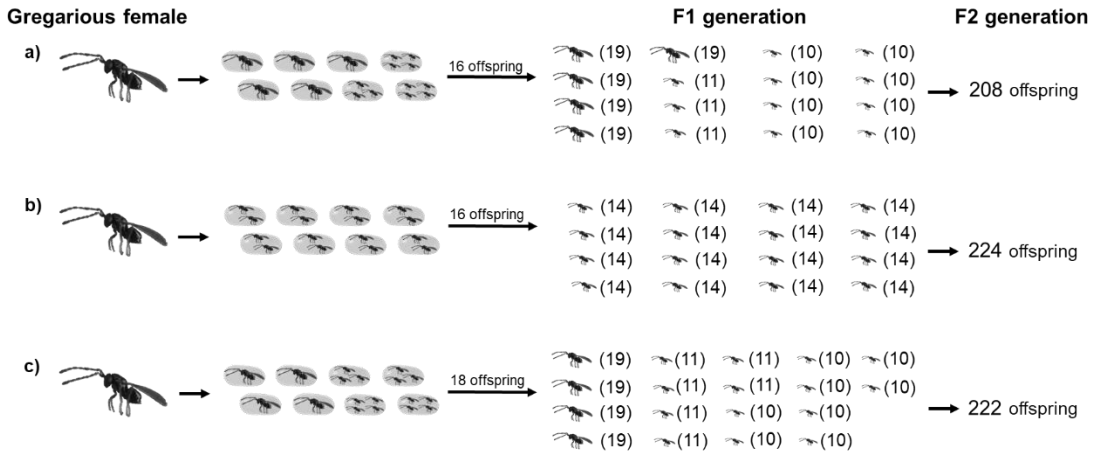

Fig. 2. The different fertility values obtained in the F2 generation of females with the same number of host eggs and the same (2a, b) or different fertility values in the F1 generation (2c) (to simplify the model, male offspring are not shown, explained in detail in the methods section *Exp. 2*).

We are aware that although the presented two-generation approach may provide a more accurate view of the population dynamics of gregarious parasitoids, determining fertility from parasitoid body size requires extensive measurements. As an alternative, we propose obtaining knowledge of factors such as host availability[11,12] and characteristics[13] or the presence of a predator of the host[11] 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 determine their population dynamics using a two-generation approach. 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, x2 = 6.824, p = 0.009; Fig. 3a, b) (this result is similar to the results of our previous study with different numbers of hosts[14]). 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, x2 = 12.193, p < 0.001; Fig. 3b), and no significant difference in clutch size was observed compared to females with 12 native hosts (LMM, x2=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). However, a two-generation approach in which parasitoid fertility is determined from body 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).

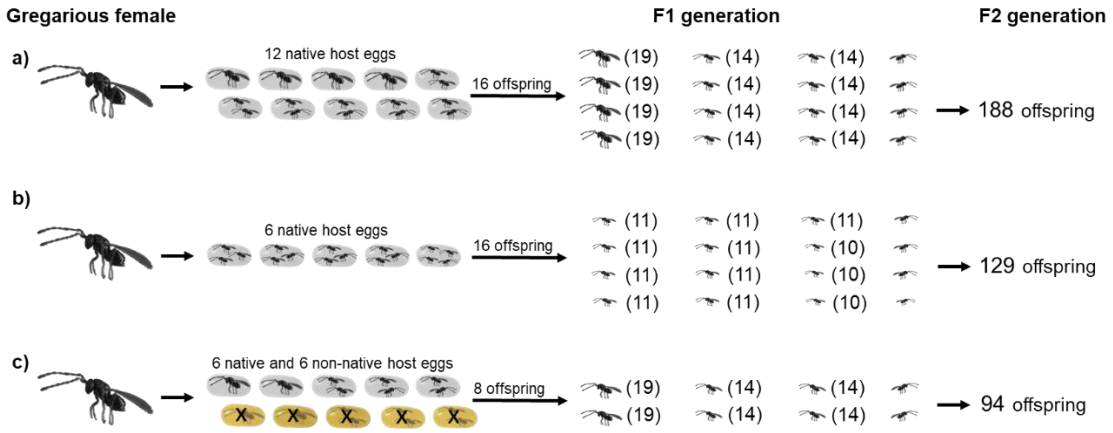

Fig. 3. The different fertility values obtained for the F2 generations of three groups of females: a) females with 12 available native host eggs; b) females with 6 available native host eggs; and c) females with 6 available native host eggs and 6 available non-native host eggs (the offspring sex ratio of *A. flavipes* is 3:1 (male:female)[8]; 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 a new two-generation approach for the determination of gregarious parasitoid population dynamics. This new approach allows more accurate results to be obtained compared to the results of the traditional approach consisting of counting the number of individuals[7,8]. Our two-generation approach may eventually allow us to assume the stability of an ecosystem more accurately or to ensure more effective uses of parasitoids in biological plant protection measures. Additionally, we propose that the reproductive strategy of gregarious parasitoids is a particularly interesting biological puzzle, as it represents a fertility-dependent and flexible life history strategy. This strategy brings benefits to its users in the form of the plasticity of clutch size combinations, by which the parasitoids can *"consciously"* or *"unconsciously"* manipulate the fertility of offspring in the future 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.6,11] and in other studies[e.g.9] because they are determined only on the basis of genital preparation[14]. 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[5].

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[5,14] 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[15].

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