Functional Ecology

British Ecological Society

Functional Ecology 2012, **26**, 1399–1408 doi: 10.1111/j.1365-2435.2012.02050.x

A quantitative framework for ovarian dynamics

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Summary

1. Ovarian dynamics describe the highly complex interplay between egg maturation, oviposition, egg resorption and the factors affecting them. While these processes have been intensively studied, further progress has been impeded by the lack of generally applicable quantitative tools to analyse experimental data.

2. The purpose of this study is to provide a quantitative framework based on a state space describing the different levels of egg maturation and resorption states (non-mature, mature, resorbing and resorbed eggs). This quantitative framework enables both the transition rates between the observable states and the unobservable states, such as resorbed eggs, to be estimated rigorously. We applied this approach to the ovarian response of the parasitoid species *Nasonia vitripennis* during a period of host and food deprivation.

3. A simple system of exclusively linear differential equations with four states captures the salient properties of the data. Resorption is shown to occur concurrently with egg maturation. We highlight widespread confusion associated with the use of the generic term 'rate of egg resorption' that has been used to describe three fundamentally different physiological processes that have contrasting rates.

4. The approach is also applied to an older data set gathered by Edwards (*The Quarterly Journal of Microscopical Science*, 1954, **95**, 459–468), which encompasses a wider range of manipulations on host and food availabilities, as well as data on oviposition. Our analysis shows that ovaries only function at full capacity after a first host meal and hints towards anautogeny in this species.

5. While this study primarily focuses on insects, the methodology is general and is suitable for studying the reproductive biology of a broad class of organisms, including plants.

Key-words: allocation strategies, egg atresia, egg maturation, egg resorption, *Nasonia vitripennis*, oogenesis

Introduction

The ovarian processes of many species are highly dynamic, responding to both individual physiological factors, such as energetic reserves or mating status, and environmental factors, such as resource availability and quality or temperature (Wheeler 1996; Papaj 2000; Nager 2006; Krysko *et al.* 2008; Vezina & Salvante 2010). At the individual level, ovarian production is regulated by the balance of two processes: oogenesis, resulting in the production of eggs that can be fertilized or laid, and oosorption (or egg resorption or egg atresia), during which eggs are destroyed and their nutrient content may be partially recovered. Throughout oogenesis, an egg goes through different stages as it undergoes different physiological processes (e.g. vitellogenesis and choriogenesis). The current state of an egg not only determines which state it will be in the near future, but it also determines an egg's sensitivity to endogenous and exogenous factors (e.g. apoptosis and endocrine factors; Terashima & Bownes 2004; Thomson, Fitzpatrick & Johnson 2010). Thus, when many eggs undergo oogenesis at the same time, the patterns of ovarian production over time not only depend on the conditions experienced by the female, but also on how eggs are distributed among states (Telfer, Gosden & Faddy 1991; Faddy & Gosden 1995; Faddy 2000). This essentially makes it a problem of structured population dynamics; therefore, any quantitative inference aiming to link conditions experienced, ovarian state and reproductive output must account for the multivariate nature of ovarian state. Most of the discussion that follows primarily focuses on the significance of these processes in insect species, but applicability of the approach is much broader and includes any species producing eggs in a dynamic fashion, including plants.

The high degree of flexibility in insect egg production has largely been interpreted as an evolutionary response to the selective pressures acting on oviposition behaviour

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(Jervis & Kidd 1986; Ellers, Sevenster & Driessen 2000; Papaj 2000; Jervis *et al.* 2001). Indeed, stochasticity in reproductive opportunities prevents a perfect match between egg production and realized reproduction (Rosenheim 1996, 2011). To the extent that egg production is costly, selection should therefore favour strategies producing the best match between these two components. Besides adjustments in foraging and oviposition behaviour, an efficient way to increase individual fitness is to finely tune ovarian production dynamically to respond rapidly to conditions experienced (Boggs 1992; Trumbo, Borst & Robinson 1995; Papaj 2000; Casas *et al.* 2009; Clifton & Noriega 2011). This can be achieved by two non-exclusive means: variable egg production and egg resorption. The functional significance of variable egg production is relatively clear: enhanced control over egg production provides a means of adaptively balancing the amounts of energy invested in reproduction and in survival (Ellers & Van Alphen 1997; Heimpel & Rosenheim 1998; Ellers, Sevenster & Driessen 2000; Jervis *et al.* 2001). In contrast, the role of egg resorption is less clear although its high prevalence among insect species suggests that it is an important one. On the other hand, the large variety of physiological mechanisms encountered among species also suggests that its significance may be variable as well (Bell & Bohm 1975). Several non-exclusive hypotheses have suggested that egg resorption may be a means of eliminating defective oocytes, maintaining continuous egg production in insects with limited storage capacity by making room for new eggs, and recovering nutrients to fuel oogenesis and/or somatic maintenance during periods of nutrient stress (King & Richards 1968; Bell & Bohm 1975; Rivero-Lynch & Godfray 1997; Nezis *et al.* 2006; Asplen 2007). Given the relatively low energy content of an egg, egg resorption is generally regarded as a poorly efficient and costly strategy to improve survival in times of nutrient stress and is thus considered to be a 'last–resort' strategy (Jervis *et al.* 2001; Bernstein & Jervis 2008). Based on an optimality approach, we recently proposed that egg resorption has a more complex involvement in foraging strategies than a simple response to starvation (Richard & Casas 2009). The inherent variability in nutrient income caused by feeding on external, stochastic sources of nutrients – such as hosts, for host-feeding parasitoids – may entail great risks of starvation, even when host availability is on average sufficient to sustain both oviposition and feeding. What distinguishes resorption from other sources of nutrients is its controllable nature. Because the reserves contained in the eggs are made readily available to the female when they are most needed, it can counteract variability in external nutrient income. In these conditions, egg resorption acts as insurance against stochasticity, and this allows females to adopt a host-use strategy with great benefits over the long term, one that would be too risky otherwise.

The rates at which the processes of egg production and resorption occur are of great importance, from ecological as well as evolutionary perspectives, as they underlie individual behavioural responses and the adaptive potential of these traits. The constraints on oviposition imposed by a finite rate of egg maturation have been regarded as sufficiently important, in terms of behavioural decisions and population dynamics, for them to be included in models (Collier 1995; Shea *et al.* 1996; Rivero & Casas 1999). Thus, the constraints on oviposition imposed by a still slower rate of resorption may then be even more severe and call for renewed attention to egg resorption (Rosenheim, Heimpel & Mangel 2000).

The study of egg resorption faces several major problems. First, we currently do not have a quantitative framework to comprehend the highly dynamic natures of egg maturation and resorption. Second, not all variables of interest and flow of energy can be observed. For example, the flow of energy from the fat body to the ovary can only be deduced at best. Also, it seems that in many insect species, the eggs are fully resorbed and do not leave any remnants that can be counted. Species leaving egg remnants in the oviducts, such as grasshoppers (Danner & Joern 2004), moths (Lum 1979) or encyrtid parasitic wasps (Lloyd 1966; Rivero-Lynch & Godfray 1997), may provide good models for studying ovarian dynamics, but in the other species, statistical inference is necessary. Alternatively, egg states may be unobservable because of the lack of experimental procedures to detect them. For example, there is currently no efficient method for characterizing eggs undergoing resorption in species that resorb fully mature and chorionated eggs, such as most hymenopteran parasitoids (Asplen 2007). Despite reports mentioning the use of vital stains, such as acetocarmine, as a common staining procedure (Jervis, Copland & Harvey 2005), it has, to our knowledge, only been reported in full in *Nasonia vitripennis* (Edwards 1954; King 1963). Given the large number of studies that dealt with egg resorption in parasitoids, this particular example seems to make it an exception rather than the rule. In *Nasonia*, resorbing eggs become increasingly permeable to vital stains, because the chorion is resorbed together with the egg (King & Richards 1968). In other species, the chorion may not be degraded during resorption (Flanders 1942; Labeyrie 1959), making the staining procedure probably ineffective.

The purpose of this study is to provide a quantitative framework accounting for the dynamic natures of egg production and resorption. The framework could then be used to rigorously estimate the rates of these processes, as well as to infer the dynamics of the different categories of the state space even if they are unobservable or cannot be determined physiologically. We applied our model to experimental data involving the ovarian dynamics of the hymenopteran *N. vitripennis* during a period of host and food deprivation, a factor known to promote oosorption. Based on this rigorous framework, we highlight widespread confusion associated with use of the generic term 'rate of egg resorption', which has been used to describe three fundamentally different physiological processes with quite different rates. It is generally unclear as to whether this term refers to the rate at which mature eggs enter into the resorptive process (e.g. Edwards 1954; King 1963; Jervis & Kidd 1986; Osawa 2005), the rate at which the remnants of completely resorbed eggs appear (e.g. Lloyd 1966; Rivero-Lynch & Godfray 1997; Danner & Joern 2004) or whether it refers to the rate at which a single egg is resorbed (e.g. McFadden, Cooper & Andersen 1965; Trepte & Trepte-Feuerborn 1980; Richard & Casas 2009). We propose a more adequate definition of these terms and quantify the different rates of egg maturation and resorption. We show the applicability of our framework by fitting an older data set of Edwards (1954), which encompasses a wider range of host- and food-deprivation periods of varying duration.

Materials and methods

EXPERIMENTAL PROCEDURES

Nasonia vitripennis (Hymenoptera: Pteromalidae) is a gregarious host-feeding ectoparasitoid of dipteran pupae (Whiting 1967). Females are synovigenic, that is, they are born with some immature eggs and can sustain egg production and maturation if sufficient nutritional resources are available (Flanders 1950; Jervis *et al.* 2001). Culturing and all experimental procedures were carried out at the Université de Tours, Institut de Recherche sur la Biologie de l'Insecte in a controlled room with constant temperature (25 °C) and constant relative humidity (75%). We also maintained insects under constant light conditions to avoid strong circadian rhythms. While these are unnatural conditions, they are routinely used as standard rearing conditions and are not known to affect *Nasonia* in any way (Werren & Loehlin 2009). Females were reared on pupae of *Phormia terrae-novae* (Diptera: Calliphoridae) obtained from a commercial manufacturer (Verminière de l'Ouest) and kept at 4 °C before use. We visually selected hosts of similar sizes, to avoid any extreme size or time trends. The parasitoid strain used (AsymCHS) was kindly provided by Prof. L. Beukeboom (University of Groningen).

The females used in the experiment came from host pupae parasitized by placing one female parasitoid among an abundance of hosts (10 hosts per female per day). To ensure full ovarian development before starting the experiment, newly emerged females were reared for the first 2 days on a diet containing a 10% (w:v) saccharose solution and with males. Females were then housed for 2 days with an abundance of hosts. The experiments were started by housing isolated females in the presence of water only. Once the deprivation period began, a group of usually 15 females (range: 12–15) was killed every 2 h by freezing them for a short period. Females were then dissected under a binocular microscope in a solution of 2% sodium sulphate. Once resorption started, the chorion became quickly permeable to vital stains (King & Richards 1968), so that resorbing eggs took up the dye and showed a deep blue coloration when stained with trypan blue. In contrast, mature eggs remained unstained as the chorion did not allow the stain to penetrate the eggs. Mature and resorbing eggs were counted after whole ovaries were stained with a solution of 0·15% trypan blue in phosphate buffered saline (PBS) for 40 min. The ovaries were washed several times with PBS.

MODEL

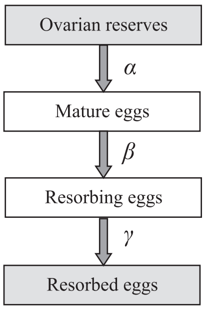
To model ovarian dynamics, we assumed that eggs could be in one of four states (Fig. 1). The first category $X(t)$, the ovarian reserves, represents either non-mature eggs or some storage of nutrients that can be used for egg maturation. This category defines the potential for egg maturation during the period of deprivation. The second category, $M(t)$, is the number of mature eggs. The third category, $R_g(t)$, is the number of eggs undergoing the process of resorption. Finally, $R_d(t)$ records the total number of eggs resorbed from the beginning of host deprivation to time t.

We divided the period of host deprivation into two periods: a period when only egg maturation takes place, and a period when maturation is accompanied by egg resorption. The transition between the two periods occurs at a time t_0. Transitions between the different categories of the state space were modelled by assuming that a constant proportion of the eggs present in one category goes into the following category at any time. The full system of equations we obtained is given in Fig. 1. The terms α can be interpreted as the specific egg maturation rate, β as the specific rate of entry in resorption and γ as the specific rate of disappearance, that is, the rate at which the nutrient content of an egg is extracted through the process of resorption. The qualifier 'specific' enables us to distinguish quantities such as the specific egg maturation rate (t^{-1}) from the egg maturation rate, which is given by $\alpha M(t)$ (eggs t^{-1}).

Fixing R_g (0) and R_d (0) to be equal to zero at the start of the experiment leads to a total of six parameters (α, β, γ, $X(0)$, $M(0)$ and t_0) to be estimated from the observation of two categories: mature and resorbing eggs.

An implicit assumption of the model is that the content of an egg undergoing resorption declines exponentially at rate γ. Theoretically, it would therefore take an infinite amount of time for the egg to be completely resorbed. We therefore report the value at which 95% of the egg content is resorbed as being the time necessary for the completion of the resorption process.

As this system is exclusively composed of linear differential equations, it was solved analytically. Parameter values were estimated from experimental data through multi-response nonlinear regressions (Bates & Watts 2007). This method, although seldom used in ecology despite its obvious interest, is a direct extension of standard univariate nonlinear regressions to systems with multiple response variables. Variance was stabilized by raising data to the power of 0·65. We chose this value because we found a power relation between the mean (μ) and standard deviation (σ) of the response variables such that $\mu \propto \sigma^{1-a}$ (mature eggs: $a = 0{\cdot}65$, $R^2 = 0{\cdot}52$; resorbing eggs: $a = 0{\cdot}66$, $R^2 = 0{\cdot}61$). Under this circumstance, it is recognized that the variance of the transformed variable will be approximately constant (Box, Hunter & Hunter 1978; Bates & Watts 2007). Residuals of the mature category were normally distributed for the whole period (Kolmogorov–Smirnov test: $D = 0{\cdot}052$, $P = 0{\cdot}12$), and those for resorbing eggs were for the period $t > t_0$ (Kolmogorov–Smirnov test: $D = 0{\cdot}044$, $P = 0{\cdot}36$). 95% confidence intervals were estimated from 1000 bootstrap replicates.



$$\frac{dX}{dt} = -\alpha X \qquad \text{eqn 1}$$

$$\frac{dM}{dt} = \begin{cases} \alpha X & \text{if } t < t_0 \\ \alpha X - \beta M & \text{if } t \geq t_0 \end{cases} \qquad \text{eqn 2}$$

$$\frac{dR_g}{dt} = \begin{cases} 0 & \text{if } t < t_0 \\ \beta M - \gamma R_g & \text{if } t \geq t_0 \end{cases} \qquad \text{eqn 3}$$

$$\frac{dR_d}{dt} = \begin{cases} 0 & \text{if } t < t_0 \\ \gamma R_g & \text{if } t \geq t_0 \end{cases} \qquad \text{eqn 4}$$

Fig. 1. Equations of the model, and diagrammatic representation of the dynamics. Grey areas represent non-observable categories of the state space. See the text for a definition of the variables and parameters.

Three models were run on the data set. The first model, presented above, is a complete model and runs over the entire time span. We also used a simplified model that was applied to the data collected only after resorption has begun, the timing of which was estimated from the complete model. By comparing two variants of this second model, with and without egg maturation, we were able to discuss the effects of concurrent egg maturation and resorption on the quality of the model fitting and on the estimated values of the parameters.

APPLYING THE FRAMEWORK ON EDWARDS' DATA

We also used the data reported by Edwards (1954) as another example of the application of our approach. With this example, we show how various experimental conditions can be accounted for through very simple modifications of the model and how to accommodate and test competing hypotheses about ovarian functioning. Details on Edwards' experimental design are reported in Fig. 2. Briefly, the data sets reported include the quantification of females' ovarian contents in the context of varying host- and food-deprivation periods as well as differing treatments before the experiments start. Edwards reported the number of eggs in five different stages: half-mature eggs; three-quarter-mature eggs; mature eggs; eggs in early stage of resorption; eggs in late stage of resorption. The first two classes are pooled in a category we refer to as non-mature eggs, $N(t)$, and the last two classes are pooled in the category resorbing eggs, $R_g(t)$. It is important to note that, unfortunately, Edwards' data are barely sufficient to yield reliable parameter estimates. The reason is that only averages of the samples are reported, sample sizes are often small, with most of them smaller than 5, and temporal resolution is relatively low, with intervals between samples being mostly greater than a day. The results of these experiments, indexed from 1 to 4 in this study, correspond to tables 4, 5, 1 and 3, respectively, in Edwards' original paper.

To analyse Edwards' experiments 1 (host- and food-deprivation treatment) and 2 (food-deprivation treatment), we extend the previous model simply by increasing the number of egg categories considered. The category $N(t)$ acts as a transitional stage between the categories $X(t)$ and $M(t)$. Its dynamics is given by the equation:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \alpha_0 X - \alpha_1 N \qquad \text{eqn 5}$$

Where α_0 is the specific transition rate from X to N, and α_1 is the specific transition rate from N to M. Equations 1 and 2 are changed accordingly, and the other equations remain unchanged.

As will be shown later, adjusting this model to Edwards' experiment 3 (honey-only treatment) does not produce a convincing fit. As an alternative model, we modify the egg maturation function by considering it to be the sum of two processes, given these experimental conditions: a declining rate of egg maturation accounting for the initially high rate of egg production as previously given by the term $\alpha N(t)$, supplemented by a small but constant rate of egg maturation α_{0C}. This assumption on the functional shape of the egg maturation process is only one possibility to improve model fit, out of many. Yet, given the current state of knowledge, it simply does not seem any less reasonable to model it this way rather than another. So, we discuss its appropriateness simply by commenting on the agreement between the model and the data.

Accounting for this additional process can be made by adding a constant α_{0C} to eqn 5, that is,

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \alpha_{0C} + \alpha_0 X - \alpha_1 N \qquad \text{eqn 6}$$

Finally, we extend the model to account for oviposition. In experiment 4 (oviposition treatment), Edwards provided females with what he qualified to be 'an ample supply of puparia' for the duration of the experiment. Under such conditions, it seems reasonable to assume host availability to be constant. Consequently, one may also assume that egg maturation proceeds at a constant rate α_{0C} only. Also, mature eggs do not enter the resorptive process anymore, but they are oviposited. As host availability is constant, we assume that mature eggs are oviposited at a rate proportional to the number of mature eggs available, $\delta M(t)$ (see Casas *et al.* 2000 for discussion of this assumption). This leads to the following system of equations:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \alpha_{0C} - \alpha_1 N \qquad \text{eqn 7}$$

$$\frac{\mathrm{d}M}{\mathrm{d}t} = \alpha_1 N - \delta M \qquad \text{eqn 8}$$

$$\frac{\mathrm{d}O}{\mathrm{d}t} = \delta M \qquad \text{eqn 9}$$

The category $O(t)$ refers to the cumulative number of eggs oviposited for the period of interest.

Note that in all the models used for analysing Edwards' experiments, the values of the rates involving egg maturation (α_0, α_1 and α_{0C}) are difficult to compare directly to the ones we estimated previously as their interpretations are different. The values of β and γ are, however, directly comparable.

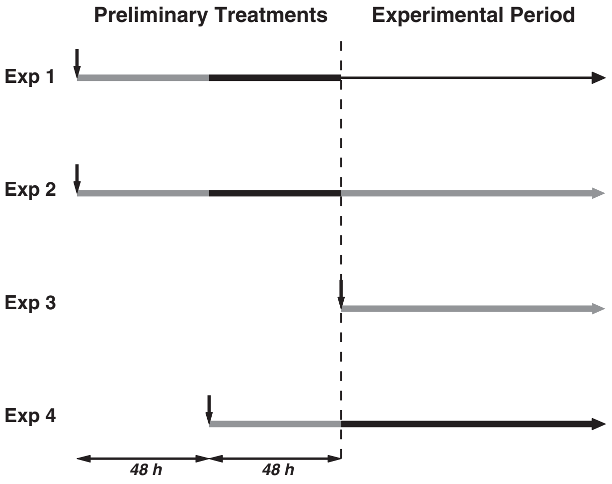


Fig. 2. Experimental design of Edwards' experiments. The different types of line indicate different diets offered to females. Thick back line: hosts present. Thick grey line: honey diet. Thin black line: host and food deprivation. We refer to the period at which the quantification of the ovarian content starts as the 'experimental period'. The beginning of this period marks the moment at which time starts (i.e. $t = 0$). Anytime before is referred to as 'preliminary treatments', and may be of different length among treatments. Therefore, females may be of different age at the beginning of the experimental period. The time of emergence is marked by an arrow. See text for further explanations.

Results

EXPERIMENTAL RESULTS

At the beginning of the period of deprivation, females had an average of 17 mature eggs. Egg load increased greatly over a short time interval and reached an average of 55 eggs after 10 h. It then steadily declined until the end of the experiment (Fig. 3b).

Egg resorption was detected in a few females as soon as 6 h after the beginning of the experiment (Fig. 4). However, egg resorption for the majority of females typically began between 10 and 12 h after the deprivation period started. The onset of egg resorption in the majority of females was consistent with the decline in egg load that began at that same time. Once resorption had started, the number of eggs being resorbed increased until about 40 h, and then declined thereafter until the end of the experiment (Fig. 3c). As this decline in the number of eggs being resorbed must be associated with the complete resorption of at least some of them, this gives an upper bound of about 30 h for an egg to be completely resorbed. As will be shown in the next section, this inference is corroborated by the value estimated from the model. The number of females having no mature eggs left started to increase from 40 h onwards (Fig. 4). This proportion stayed generally below 50% until the end of the experiment.

FIT OF THE COMPLETE MODEL

Model predictions are in relatively good agreement with experimental data (Fig. 3). Estimated parameter values are given in Table 1. Females are predicted to start resorbing eggs after 10·4 h of deprivation. A total of 42·7 eggs were resorbed over the course of the experiment, whereas a total of 27·7 eggs were matured, including 7·5 that matured during the period in which egg resorption was taking place. The value reported for the parameter γ indicates that it takes 31·6 h for 95% of the content of one egg to be resorbed.

CONCURRENT EGG MATURATION AND EGG RESORPTION

To estimate the non-zero egg maturation rate during the period when eggs are undergoing resorption, we refitted the data for the period when resorption was occurring ($t \geq t_0$), and compared the goodness of fit of the simplified model either with maturation or without maturation. The gain in goodness of fit was small (negative log-likelihood = 1028·2 vs. 1030·4). As the model without maturation contains two fewer parameters, neither model was statistically preferred (ΔAIC = 0·4). Nonetheless, the model with egg maturation predicts a substantial amount of egg maturation (about 11 eggs, Table 1). Noteworthy, 95% of egg maturation occurred within 10 h after the onset of egg resorption. The simultaneous occurrence of egg maturation and resorption is therefore substantial only during early stages of resorption. Both of the simplified models predict resorption rates similar to those predicted by the full model (Table 1). Overall, the inclusion or exclusion of egg maturation in the model resulted in slightly different predictions for the number of eggs being

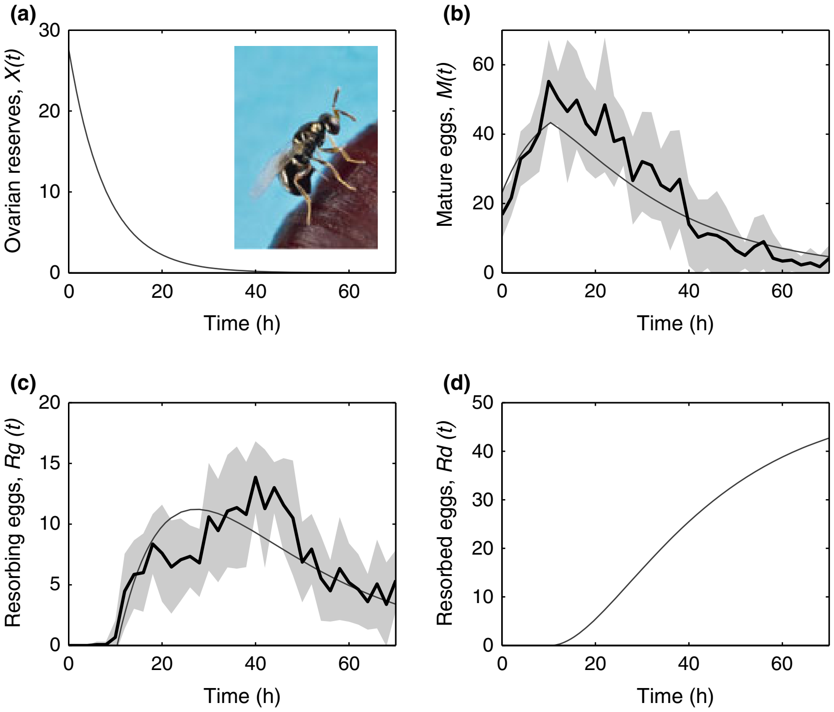


Fig. 3. Ovarian dynamics of (a) Non-mature eggs, (b) Mature eggs, (c) Resorbing eggs, (d) Resorbed eggs. Thin lines are model predictions, black lines are experimental averages and grey areas indicate the region defined by the mean ± SD. Note that $t = 0$ indicates the time at which measurements began. Females are therefore 4 days old at that time. The photograph in (a) shows a female *Nasonia vitripennis* ovipositing on a host pupa. Photograph taken by Peter Koomen.

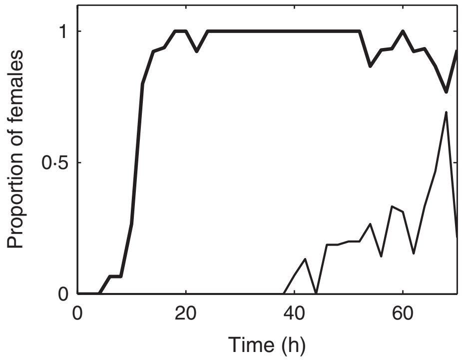


Fig. 4. Observed proportion of females with resorbing eggs (thick black line), and no mature egg at all (thin grey line). Note that $t = 0$ indicates the time at which measurements began. Females are therefore 4 days old at that time.

resorbed. With egg maturation, 44·3 eggs were resorbed, whereas 41 eggs were resorbed without egg maturation. This resulted in a difference of about 8%.

EDWARDS' EXPERIMENTS

The parameter estimates for all of Edwards' experiments are provided in Table 2, and experimental data and the model predictions are presented in Figs 5 and 6.

Edwards' first experiment (host and food deprivation) includes only four data points, which makes any use of these data hardly reliable. Nonetheless, despite some quantitative differences in the number of mature eggs present in females' ovaries, egg maturation and resorption patterns seem similar to those reported in our experiment (compare Figs 5a–e to 3). The estimate of β is slightly higher and the estimate of γ is similar to our estimates reported in Table 1, which is quite a good agreement given the very reduced number of data points in Edwards' experiment.

Applying the framework to Edwards' second experiment (food deprivation) produces a very good agreement between model predictions and experimental data (Fig. 5f–j). The examination of parameter estimates reveals that females provided with a diet of honey maintained a substantially higher rate of egg maturation from reserves compared with his first treatment (best seen in Fig. 5f). Also, this produced a much lower value for β, while γ and α_1 remain very close. This suggests that a honey diet affects mostly the rate at which energy is invested in ovaries, and the rate at which mature eggs enter the resorptive process, but not the time it takes for an egg to be matured, or to be resorbed.

For Edwards' third experiment (honey only), the model previously used does not successfully capture the trend in $N(t)$ over the long run (Fig. 5l, dashed line). Essentially, no egg is expected in this category after 200 h, which does not agree well with data. This mismatch is caused by the decline in egg maturation rate implied by the finite amount of energy that can be invested in egg maturation, $X(0)$. The improved model – including a constant component to the egg maturation rate (eqn 6) – accounts much better for the temporal pattern of the category $N(t)$ (Fig. 5l, continuous line). This modification affects parameter estimates substantially (Table 2), and also the dynamics of the other categories (Fig. 5l–o), but it is hard to tell whether this constitutes an improvement overall or not. Neither model is to be preferred on statistical ground (ΔAIC = 0·007).

The good agreement between the model and data from Edwards' fourth experiment (oviposition treatment) shows that the dynamics in ovarian production and oviposition can be accounted for quite simply under conditions of constant host availability (Fig. 6), during which females laid an average of 50 eggs per day (Fig. 6c).

Discussion

Current understanding of ovarian dynamics in insects and other arthropods has relied heavily on various manipulations of host and food availability (Bell & Bohm 1975; Papaj 2000). Nonetheless, the absence of an archetypal model prevents making a comprehensive account of the processes involved in ovarian dynamics and does not allow them to be quantified adequately. The models presented in this study provide a simple picture of these processes, and may prevent possible confusion arising from analyses of

Table 1. Parameter estimates for the full model and the simplified models

		Full model		Simplified models	
		95% bootstrap confidence interval			
Parameter	Estimate	Lower bound	Upper bound	With egg maturation	Without egg maturation
α	0·1261	0·1224	0·1462	0·2285	–
β	0·0412	0·0405	0·0424	0·0418	0·0439
γ	0·0947	0·0924	0·0969	0·0981	0·0916
t_0	10·39	10·29	11·00	–	–
$X(0)$	27·70	26·89	28·59	–	–
$M(0)$	23·07	22·22	23·52	–	–
$X(t_0)$	–	–	–	10·97	–
$M(t_0)$	–	–	–	41·21	49·71

Table 2. Applying our framework to Edwards' experiments. The design of the experiments is given in Fig. 2. Note that females are of different age at the beginning of the experiments

Experiment #	1	2	3 (first model)	3 (second model)	4
Parameter					
α_{0C}	–	–	–	0·0732	1·9780
α_0	0·0230	0·0033	0·8184	0·1789	–
α_1	0·0981	0·1084	0·0148	0·0221	–
β	0·0737	0·0123	0·0025	0·0081	–
γ	0·1027	0·1218	0·0087	0·0388	–
δ	–	–	–	–	0·1622
t_0	13·35	0	48·00	70·34	–
$X(0)$	23·62	167·24	24·29	22·99	–
$N(0)$	24·11	22·45	–	–	9·68
$M(0)$	10·03	8·473	–	–	16·60

such data. In the discussion that follows, we address the implementation of the model to the specific biology of *Nasonia* and conclude by discussing the generality of our framework.

APPLYING THE FRAMEWORK TO *NASONIA*

Our estimates of egg resorption and egg maturation rates for this species match well with the estimates made in previous studies. The time necessary to resorb an egg, previously inferred to take between 1 and 2 days (King & Richards 1968), was given a value of 31·6 h in our study. The analysis of Edwards' experimental data suggests that honey diet affects mostly the rate at which energy is invested in ovaries, and the rate at which mature eggs enter the resorptive process, but not the time it takes for an egg to be matured, or to be resorbed. Nonetheless, the ovarian dynamics experienced during a period of host and/or food deprivation may be critically influenced by previous history of the female, particularly in early adult life. Indeed, the ovarian dynamics of females subjected to a period of host deprivation from emergence were much different from those of females that encountered hosts in early adult life, with egg maturation and resorption operating at slow motion (compare the results from Edwards' second and third experiments). This suggests that *Nasonia* are partly anautogenic, that is, the full potential of the ovarian machinery is unleashed only after a first meal on a host.

AN ARCHETYPAL MODEL FOR THE ANALYSIS OF OVARIAN DYNAMICS

The term 'egg resorption rate' is used widely in the study of ovarian dynamics. As we mentioned in the introduction, it is generally unclear whether this term refers to the rate at which mature eggs enter into the resorption process, the rate at which the remnants of completely resorbed eggs appear, or thirdly, whether it refers to the rate at which a single egg is resorbed. In our model, these different concepts correspond to $\beta M(t)$, $\gamma R_g(t)$ and γ, respectively. Thus, different uses of the term 'egg resorption rate' obscure the fact that two basic rates, β and γ, are involved, and not just one. A comparison of the two rates shows that they are quite different, with the first being twice as high as the second. Thus, eggs enter into the resorptive pathway at a much higher rate than the rate at which they are actually resorbed. Different conclusions about the speed of egg resorption might be drawn if these two rates are confused or applied incorrectly. The lesson here is that estimating the timing and flow of energy requires a proper definition of the state space, and any failure to do so will lead to erroneous estimates of ovarian processes. For example, neglecting the importance of transitional resorbing state, which has been done in other studies, not only generates sensitive errors in the context of an energy budget (Rivero & Casas 1999; Casas *et al.* 2005; Kooijman 2010) but is also surprising in the light of the care given to the estimation and modelling of egg maturation rates (Casas *et al.* 2000; Wang & Messing 2003; Wu & Heimpel 2007).

While the determinants of ovarian dynamics may be largely different in other classes of organisms, it remains true that eggs undergo a series of transformations before being fully mature or fully resorbed. This is true for most metazoans, and even plant species (Matova & Cooley 2001; de Jong & Klinkhamer 2005; Lubzens *et al.* 2010). In all instances, a tremendous variety of experiments have been conduced to understand both normal ovarian functioning and its response to biological factors and chemical compounds. The largest class of such experiments involves the comparison between a control and some experimental groups. This is classically analysed through pairwise comparisons or analyses of variance on the different stage class of eggs, potentially at different times (e.g. Wood & Van Der Kraak 2001; Terashima & Bownes 2004, Viana *et al.* 2010). While such analyses can deal correctly with the total effect of treatments on reproductive output, they barely establish any mechanistic link between these two components. A comparison between the values of measures reflecting ovarian functioning in a comprehensive way, such as rates, is better suited to meet these goals. They could be directly estimated using the quantitative tools presented in this study. As we illustrated, such an approach also has the advantageous possibility of circumventing some experimental handicaps by allowing predictions to be made on unobservable states.

The basic model presented in this study includes some of the simplest assumptions about ovarian functioning. We provided further examples of model extensions by applying the framework to Edwards' data set, in which *Nasonia* females were subjected to various experimental conditions. Only a few modifications of the model were necessary to produce a good agreement between models and data. This is good news for behavioural and population ecologists using physiologically structured models, and we hope these few examples will provide a source of inspiration for further work involving different organisms in different contexts.

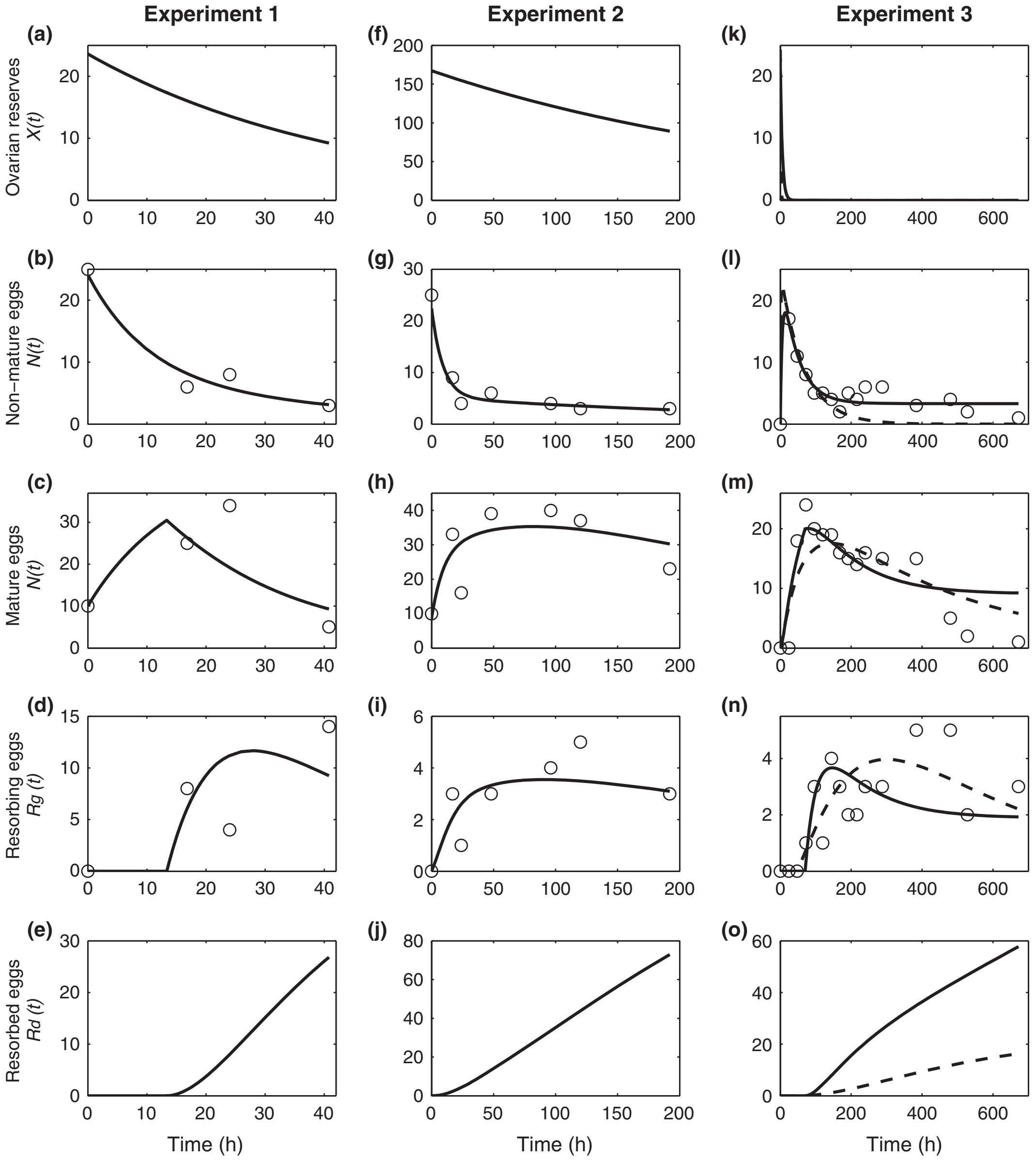


Fig. 5. Ovarian dynamics and model prediction for Edwards' experiments numbered 1–3. Lines are model predictions, and circles are Edwards' data. The dashed lines in the graphs of experiment 3 correspond to the first model tested; the continuous line corresponds to the second model, with a constant component added to egg maturation rate.

Further development of this model from a physiological perspective could be made by moving from constant specific transition rates to more realistic time- or state-dependent rates. For example, the probability of a mature egg entering into the resorptive process (β) might increase as a function of the time since the beginning of a deprivation period. Accounting for the effect of stochasticity in those ecological factors that affect ovarian dynamics, such as host and food in parasitoids, is another line of improvement of our model, from a behavioural and ecological perspective (Papaj 2000). When these factors are stochastic, the ovarian dynamics will depend on the specific sequence of host and food encounter. Extending the approach to such circumstances requires two additional ingredients. First, one needs to have a bookkeeping of these events by including variables such as the time since the last host feeding, since the last sugar feeding, since the last oviposition or the amount of nutrient reserves. For example, if sugar feeding is unavailable, the ovarian dynamics between two host encounters may be simply considered as a period of host and food deprivation, and the ovarian dynamics between these events can be predicted by using a model parameterized under these conditions. Second, one needs to account for the interaction between the effects of these variables on ovarian dynamics. The real challenge may not lie in the greater mathematical complexity of adding more realism to this model, but in obtaining experimental evidence for its inclusion.

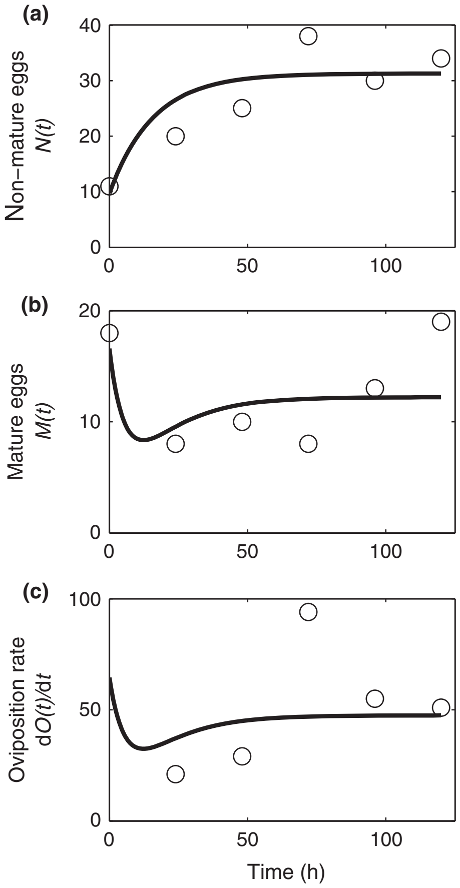


Fig. 6. Ovarian dynamics and model prediction for Edwards' experiment 4.

Acknowledgements

We are grateful to Leo Beukeboom for kindly providing the *Nasonia* individuals we used in this study. We also thank one of the referees for pushing us to analyse Edwards' data, as well as providing sharp comments on an earlier version of this manuscript. David Giron, Paul Simpson and two referees made valuable comments that improved the manuscript. This work is based on the Master's thesis of R. Richard, carried out at the University of Tours. Support for this research was provided by the University of Tours and the CNRS.

References

Asplen, M.K. (2007) *Life History Correlates in Female Parasitoid Wasps: Exploring Old Concepts and New Frontiers*. University of Arizona, Tucson, Arizona, USA.

Bates, D.M. & Watts, D.G. (2007) *Nonlinear Regression Analysis and Its Applications*. John Wiley & Sons, New York, New York, USA.

Bell, W.J. & Bohm, M.K. (1975) Oosorption in insects. *Biological Reviews*, **50**, 373–396.

Bernstein, C. & Jervis, M.A.. (2008) Food searching in parasitoids: the dilemma of choosing between "immediate" or future fitness gains. *Behavioral Ecology of Insect Parasitoids: From Theoretical Approaches to Field Applications* (eds É. Wajnberg, C. Bernstein & J. van Alphen), pp. 129–171. Blackwell Publishing, London.

Boggs, C.L. (1992) Resource allocation: exploring connections between foraging and life history. *Functional Ecology*, **6**, 508–518.

Box, G.E.P., Hunter, J.S. & Hunter, W.G. (1978) *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building*. John Wiley & Sons, New York, New York, USA.

Casas, J., Nisbet, R.M., Swarbrick, S. & Murdoch, W.W. (2000) Eggload dynamics and oviposition rate in a wild population of a parasitic wasp. *Journal of Animal Ecology*, **69**, 185–193.

Casas, J., Pincebourde, S., Mandon, N., Vannier, F., Poujol, R. & Giron, D. (2005) Lifetime nutrient dynamics reveal simultaneous capital and income breeding in a parasitoid. *Ecology*, **86**, 545–554.

Casas, J., Vannier, F., Mandon, N., Delbecque, J.P., Giron, D. & Monge, J. P. (2009) Mitigation of egg limitation in parasitoids: immediate hormonal response and enhanced oogenesis after host use. *Ecology*, **90**, 537–545.

Clifton, M.E. & Noriega, F.G. (2011) Nutrient limitation results in juvenile hormone-mediated resorption of previtellogenic ovarian follicles in mosquitoes. *Journal of Insect Physiology*, **57**, 1274–1281.

Collier, T.R. (1995) Adding physiological realism to dynamic state variable models of parasitoid host feeding. *Evolutionary Ecology*, **9**, 217–235.

Danner, B.J. & Joern, A. (2004) Development, growth, and egg production of *Ageneotettix deorum* (Orthoptera: Acrididae) in response to spider predation risk and elevated resource quality. *Ecological Entomology*, **29**, 1–11.

Edwards, R.L. (1954) The effect of diet on egg maturation and resorption in *Mormoniella vitripennis* (Hymenoptera, Pteromalidae). *The Quarterly Journal of Microscopical Science*, **95**, 459–468.

Ellers, J., Sevenster, J.G. & Driessen, G. (2000) Egg load evolution in parasitoids. *The American Naturalist*, **156**, 650–665.

Ellers, J. & Van Alphen, J.J.M. (1997) Life history evolution in *Asobara tabida*: plasticity in allocation of fat reserves to survival and reproduction. *Journal of Evolutionary Biology*, **10**, 771–785.

Faddy, M.J. (2000) Follicle dynamics during ovarian ageing. *Molecular and Cellular Endocrinology*, **163**, 43–48.

Faddy, M.J. & Gosden, R.G. (1995) A mathematical model of follicle dynamics in the human ovary. *Human Reproduction*, **10**, 770–775.

Flanders, S.E. (1942) Oösorption and ovulation in relation to oviposition in the parasitic hymenoptera. *Annals of the Entomological Society of America*, **6**, 251–266.

Flanders, S.E. (1950) Regulation of ovulation and egg disposal in the parasitic hymenoptera. *The Canadian Entomologist*, **82**, 134–140.

Heimpel, G.E. & Rosenheim, J.A. (1998) Egg limitation in parasitoids: a review of the evidence and a case study. *Biological Control*, **11**, 160–168.

Jervis, M.A., Copland, M.J.W. & Harvey, J.A.. (2005) The life cycle. *Insects as Natural Enemies* (ed. M.A. Jervis), pp. 74–165. Springer, Dordrecht.

Jervis, M.A. & Kidd, N.A.C. (1986) Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews*, **61**, 395–434.

Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A. & Kidd, N.A.C. (2001) Life-history strategies in parasitoid wasps: a comparative analysis of "ovigeny". *Journal of Animal Ecology*, **70**, 442–458.

de Jong, T.J. & Klinkhamer, P.G.L. (2005) *Evolutionary Ecology of Plant Reproductive Strategies*. Cambridge University Press, Cambridge, UK.

King, P.E. (1963) The rate of egg resorption in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) deprived of hosts. *Proceedings of the Royal Entomological Society of London. Series A, General Entomology*, **38**, 98–100.

King, P.E. & Richards, J.G. (1968) Oösorption in *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Journal of Zoology*, **154**, 495–516.

Kooijman, B. (2010) *Dynamic Energy Budget Theory for Metabolic Organisation*, 3rd edn. Cambridge University Press, Cambridge, UK.

Krysko, D.V., Diez-Fraile, A., Criel, G., Svistunov, A.A., Vandenabeele, P. & D'Herde, K. (2008) Life and death of female gametes during oogenesis and folliculogenesis. *Apoptosis*, **13**, 1065–1087.

Labeyrie, V. (1959) Sur l'origine de la résorption des oeufs chez *Diadromus pulchellus* (Hyménoptère, Ichneumonidae), à la lumière d'un cas anormal de développement embryonnaire. *Comptes rendus hebdomadaires des séances de l'Académie des sciences*, **249**, 2115–2117.

Lloyd, D.C. (1966) Some aspects of egg resorption in *Leptomastix Dactylopii* how [Hymenoptera, Encyrtidae]. *Entomophaga*, **11**, 365–381.

Lubzens, E., Young, G., Bobe, J. & Cerdà, J. (2010) Oogenesis in teleosts: how eggs are formed. *General and Comparative Endocrinology*, **165**, 367–389.

Lum, P.T.M. (1979) Degeneration of ova in the bulla seminalis of Lepidoptera. *Journal of Insect Physiology*, **25**(595–597), 599.

Matova, N. & Cooley, L. (2001) Comparative aspects of animal oogenesis. *Developmental Biology*, **231**, 291–320.

McFadden, J.T., Cooper, E.L. & Andersen, J.K. (1965) Some effects of environment on egg production in brown trout (*Salmo trutta*). *Limnology and Oceanography*, **10**, 88–95.

Nager, R.G. (2006) The challenges of making eggs. *Ardea*, **94**, 323–346.

Nezis, I.P., Stravopodis, D.J., Margaritis, L.H. & Papassideri, I.S. (2006) Follicular atresia during *Dacus oleae* oogenesis. *Journal of Insect Physiology*, **52**, 282–290.

Osawa, N. (2005) The effect of prey availability on ovarian development and oosorption in the ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). *European Journal of Entomology*, **102**, 503–511.

Papaj, D.R. (2000) Ovarian dynamics and host use. *Annual Review of Entomology*, **45**, 423–448.

Richard, R. & Casas, J. (2009) Stochasticity and controllability of nutrient sources in foraging: host-feeding and egg resorption in parasitoids. *Ecological Monographs*, **79**, 465–483.

Rivero, A. & Casas, J. (1999) Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. *Researches on Population Ecology*, **41**, 39–45.

Rivero-Lynch, A.P. & Godfray, H.C.J. (1997) The dynamics of egg production, oviposition and resorption in a parasitoid wasp. *Functional Ecology*, **11**, 184–188.

Rosenheim, J.A. (1996) An evolutionary argument for egg limitation. *Evolution*, **50**, 2089–2094.

Rosenheim, J.A. (2011) Stochasticity in reproductive opportunity and the evolution of egg limitation in insects. *Evolution*, **65**, 2300–2312.

Rosenheim, J.A., Heimpel, G.E. & Mangel, M. (2000) Egg maturation, egg resorption and the costliness of transient egg limitation in insects. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **267**, 1565–1573.

Shea, K., Nisbet, R.M., Murdoch, W.W. & Yoo, H.J.S. (1996) The effect of egg limitation on stability in insect host-parasitoid population models. *Journal of Animal Ecology*, **65**, 743–755.

Telfer, E., Gosden, R.G. & Faddy, M.J. (1991) Impact of exogenous progesterone on ovarian follicular dynamics and function in mice. *Journal of Reproduction and Fertility*, **93**, 263–269.

Terashima, J. & Bownes, M. (2004) Translating available food into the number of eggs laid by Drosophila melanogaster. *Genetics*, **167**, 1711–1719.

Thomson, T.C., Fitzpatrick, K.E. & Johnson, J. (2010) Intrinsic and extrinsic mechanisms of oocyte loss. *Molecular Human Reproduction*, **16**, 916–927.

Trepte, H.-H. & Trepte-Feuerborn, C. (1980) Development and physiology of follicular atresia during ovarian growth in the house fly, *Musca domestica*. *Journal of Insect Physiology*, **26**, 329–338.

Trumbo, S.T., Borst, D.W. & Robinson, G.E. (1995) Rapid elevation of juvenile hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology*, **41**, 535–543.

Vezina, F. & Salvante, K.G. (2010) Behavioral and physiological flexibility are used by birds to manage energy and support investment in the early stages of reproduction. *Current Zoology*, **56**, 767–792.

Viana, J.H.M., Palhao, M.P., Siqueira, L.G.B., Fonseca, J.F. & Camargo, L.S.A. (2010) Ovarian follicular dynamics, follicle deviation, and oocyte yield in Gyr breed (Bos indicus) cows undergoing repeated ovum pick-up. *Theriogenology*, **73**, 966–972.

Wang, X.-G. & Messing, R.H. (2003) Egg maturation in the parasitoid *Fopius arisanus* (Hymenoptera: Braconidae): do host-associated stimuli promote ovarian development? *Annals of the Entomological Society of America*, **96**, 571–578.

Werren, J.H. & Loehlin, D.W. (2009) Strain Maintenance of *Nasonia vitripennis* (Parasitoid Wasp). *Cold Spring Harbor protocols*, **2009**, pdb. prot5307.

Wheeler, D. (1996) The role of nourishment in oogenesis. *Annual Review of Entomology*, **41**, 407–431.

Whiting, A.R. (1967) The biology of the parasitic wasp *Mormoniella vitripennis* [=*Nasonia brevicornis*] (Walker). *The Quarterly Review of Biology*, **42**, 333–406.

Wood, A.W. & Van Der Kraak, G.J. (2001) Apoptosis and ovarian function: novel perspectives from the teleosts. *Biology of Reproduction*, **64**, 264–271.

Wu, Z. & Heimpel, G.E. (2007) Dynamic egg maturation strategies in an aphid parasitoid. *Physiological Entomology*, **32**, 143–149.

Received 12 January 2012; accepted 10 July 2012
Handling Editor: Peter Mayhew