Reproductive asynchrony in natural butterfly populations and its consequences for female matelessness

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Summary

1. Reproductive asynchrony, where individuals in a population are short-lived relative to the population-level reproductive period, has been identified recently as a theoretical mechanism of the Allee effect that could operate in diverse plant and insect species. The degree to which this effect impinges on the growth potential of natural populations is not yet well understood.
2. Building on previous models of reproductive timing, we develop a general framework that allows a detailed, quantitative examination of the reproductive potential lost to asynchrony in small natural populations.
3. Our framework includes a range of biologically plausible submodels that allow details of mating biology of different species to be incorporated into the basic reproductive timing model.
4. We tailor the parameter estimation methods of the full model (basic model plus mating biology submodels) to take full advantage of data from detailed field studies of two species of *Parnassius* butterflies whose mating status may be assessed easily in the field.
5. We demonstrate that for both species, a substantial portion of the female population (6·5–18·6%) is expected to die unmated. These analyses provide the first direct, quantitative evidence of female reproductive failure due to asynchrony in small natural populations, and suggest that reproductive asynchrony exerts a strong and largely unappreciated influence on the population dynamics of these butterflies and other species with similarly asynchronous reproductive phenology.

Key-words: Allee effect, female reproductive success, mating behaviour, *Parnassius clodius*, *P. smintheus*, reproductive asynchrony.

Introduction

The Allee effect, where population growth rate decreases at low population densities, is recognized increasingly as a significant feature of many species' population dynamics (McCarthy 1997; Courchamp, Clutton-Brock & Grenfell 1999; Stephens & Sutherland 1999; Dennis 2002). It is now accepted widely that understanding how Allee dynamics arise from species' life-history traits is both a fundamental research goal and a high priority for conservation efforts (Courchamp *et al*. 1999; Stephens & Sutherland 1999). Allee effects have many causes (Courchamp *et al*. 1999), but typically the proximate mechanism is decreased mate-finding efficiency at low popu-

lation densities (McCarthy 1997; Wells *et al*. 1998). In populations subject to mate-finding Allee effects, females may either have lower realized fecundity at low density or may fail to mate altogether. While problems of spatial mate location are well studied (Veit & Lewis 1996; McCarthy 1997; Wells *et al*. 1998; Boukal & Berec 2002), the related problem of finding mates when individuals have limited temporal overlap with each other has received much less attention.

Recently, Calabrese & Fagan (2004; hereafter C & F) demonstrated that populations featuring asynchronous reproduction may be subject to a temporal Allee effect. Reproductive asynchrony, when individuals within a population do not overlap completely in time, is most important in strongly seasonal populations with a defined breeding period (e.g. univoltine butterflies). In many species, individuals enter the

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breeding population at different times during the mating season, whether by asynchronous emergence from a juvenile or overwintering stage, or by asynchronous arrival at preferred breeding sites. Such asynchrony is exacerbated by individuals having variable and often short residency times within the breeding population. Note that the definition of asynchrony does not require that average emergence/arrival times differ between the sexes. Previous theoretical studies have found that asynchrony can be an effective bet-hedging strategy in the face of environmental unpredictability (Iwasa 1991; Iwasa & Levin 1995). However, these studies did not consider density-dependent mate-finding issues explicitly when populations decline. In contrast, C & F used a simple model to demonstrate that the average individual in an asynchrous population, being reproductively active for only a portion of the breeding season, has fewer mating opportunities relative to the average individual in a more synchronous population of the same size. This decreased temporal overlap with potential mates translates into an increasing proportion of females failing to mate when asynchrony increases in populations of fixed size or when population size decreases in populations with a fixed degree of asynchrony. Their results suggest that, although asynchrony may be an advantageous bet-hedging strategy in high-density populations, it can be costly at low density.

Protandry, where males emerge on average before females, is a phenological trait that often accompanies asynchrony. Most previous work on protandry has, as with asynchrony, been directed at determining benefits. Both males and females can profit from protandry, although the optimal amount of protandry may differ between the sexes and, in general, decreases with decreasing population density (Wiklund & Fagerström 1977; Zonneveld & Metz 1991). C & F showed that protandry may work in concert with asynchrony to exacerbate Allee effects in declining populations, but that protandry's contribution is relatively small. We therefore consider asynchrony *per se* to be the main driver of phenological Allee effects, but we acknowledge that these two factors can be difficult to separate in practice.

Our goal here is to quantify the negative effects of asynchrony in low-density populations. Despite its conceptual utility, the C & F model is not an appropriate tool for doing so because: (1) as an individual-based stochastic simulation, the C & F model is difficult to fit directly to data; and (2) the probability that two opposite-sex individuals mate is modelled as the realized proportion of their potential temporal overlap, based on an assumption of constant, equal-length individual life spans. These assumptions leave no room for a more realistic treatment of the details of mating biology (hereafter 'mating factors'), which vary, often strikingly, among species and which may directly alter the population impact of a given amount of asynchrony. For example, if individuals in animal populations increase mate-searching efforts actively when mates are rare (Kokko & Rankin 2006), then some negative effects of asynchrony might be avoided. Other examples include context-dependent variation in female choice (Kokko & Mappes 2005; Kokko & Rankin 2006) and age- or size-dependent male reproductive success (Kemp, Wiklund & Gotthard 2006). A more general and flexible approach, which accommodates species-specific mating factors and connects more directly with data, is needed to quantify asynchrony's negative effects in real populations.

To do so, we build on the framework of the Zonneveld model (Zonneveld & Metz 1991; Zonneveld 1991, 1992; 1996a,b), which can be fitted to data in a straightforward manner via maximum likelihood. The Zonneveld model focuses upon within-breeding-season population dynamics by coupling equations describing the change in abundance of reproductively mature males and females over time with a kinetic-based mating equation. This latter feature can be generalized to incorporate alternative mating factors into the model. A set of alternative models, each embodying a different mating factor hypothesis, can then be built. Connecting such models to data requires an estimate of female reproductive success, which can be obtained for many taxa using experimental or observational approaches (e.g. Burns 1968; Augspurger 1981; Bertin & Cezilly 2005; Duncan *et al.* 2004). The set of resulting models incorporating different mating factor hypotheses, each appropriately parameterized, can then: (1) predict the season-long proportion of the female population that dies without mating; and (2) draw inference on the features of the mating system that govern reproductive dynamics.

Here, we quantify the reproductive potential lost to asynchrony in low-density populations of the butterflies *Parnassius clodius* Menetries and *P. smintheus* Doubleday. We take advantage of an important feature of *Parnassius* reproductive biology; namely, that after mating a male deposits a visually obvious structure (the sphragis or mating plug) on the female's abdomen that prevents her from remating (Scott 1986). The mating status of female *Parnassius* can therefore be assessed directly and non-lethally in the field, permitting estimation of female mating efficiency throughout the breeding season. To extract this information, we extend the above-mentioned maximum likelihood methods to directly use data on unmated females, and employ a comparison framework to rank the mating factor submodels for each species. We then use the parameterized models to predict the proportion of females that die without mating and relate this to the populations' degree of asynchrony. For populations for which insufficient data precluded these analyses, we use logistic regression to characterize the timing of male and female reproductive activity and the proportion of unmated females across the breeding season. These results are then compared, within species, to those of the full analyses. We find that, for both species, a considerable proportion of females is expected to die before mating, implying a lower limit on each species' growth rate that must be exceeded to avoid an Allee effect. We conclude by discussing the potential of our approach to unify studies of the consequences of asynchrony across taxa.

Methods

BASIC REPRODUCTIVE TIMING MODEL

We define an asynchrony model based on three fundamental characteristics: (1) individuals must, on average, be available to mate for

only a fraction of the population-level breeding period; (2) females are not guaranteed to mate before they die; and (3) population density of both males and females must be modelled explicitly throughout the reproductive activity period. Other well-known evolutionary models of reproductive phenology do not qualify as asynchrony models for ecological dynamics because they lack at least one of these features (e.g. Wiklund & Fagerström 1977; Iwasa 1991). Although the Zonneveld model is an asynchrony model by the above criteria, it was developed and analysed strictly in the context of the evolution of protandry, leaving its consequences for asynchrony unexplored.

Assuming non-overlapping generations and no net flux of individuals due to immigration and emigration, the rate of change in the density of reproductively mature males (M) and females (F: this includes mated and unmated females) is a balance between emergence rate of new adults from the pupal stage and the death rate of existing adults:

$$\frac{dM}{dt} = M_0 g(t, \theta_m) - \alpha_m M \qquad \text{eqn 1a}$$

$$\frac{dF}{dt} = F_0 g(t, \theta_f) - \alpha_m F \qquad \text{eqn 1b}$$

where t is time (days), M_0 and F_0 are total densities of males and females, respectively, $g(t, \theta)$ is a probability distribution, with parameter vector θ, dictating how emergence events are spread over time, and α is a constant, per-day death rate. The subscripts m and f allow parameters to differ between males and females. This is the same general emergence and death model described originally by Manly (1974) and elaborated by Zonneveld (1991). Importantly, this model has well-established parameter estimation methods (Zonneveld 1991; Longcore *et al.* 2003; Gross *et al.* 2007).

C & F showed that the shape of the emergence distribution, which is usually not known in advance, could modulate the effects of asynchrony. We therefore chose the gamma distribution because it is flexible in shape but relatively parameter-sparse. The gamma probability density function is:

$$g(t, \theta) = \frac{\lambda}{\Gamma(\mu)} (\lambda t)^{\mu-1} \text{Exp}(-\lambda t) \qquad \text{eqn 2}$$

where $\theta = (\lambda, \mu)$, λ is the inverse scale parameter, μ is the shape parameter and $\Gamma(\cdot)$ is the gamma function.

We use the standard and widely applicable kinetic approach to describe mate encounter, assuming that the number of matings per unit time is proportional to the product of male and unmated female density (Wiklund & Fagerström 1977; Wells, Wells & Cook 1990; Zonneveld & Metz 1991; Zonneveld 1992; 1996a,b; Wells *et al.* 1998; Hutchinson & Waser 2007). As in C & F, we assume that females are monandrous and that males may mate many times. The rate of change in the density of unmated females, denoted U, is then

$$\frac{dU}{dt} = F_0 g(t, \theta_f) - c(\cdot)MU - \alpha_f U \qquad \text{eqn 3}$$

where $c(\cdot)$ is, in general, a function representing the instantaneous mating rate (efficiency) and captures species-specific details of mating biology (see below). The cumulative density of mated females at any time $R(t)$, is given by the solution to:

$$\frac{dR}{dt} = c(\cdot)MU. \qquad \text{eqn 4}$$

For a given parameter set, the proportion of mateless females can be calculated as:

$$q(t) = 1 - \frac{R(t)}{F_0}. \qquad \text{eqn 5}$$

As we are interested in the total, season-long proportion of females that die mateless, we evaluate $q(t)$ for large t (i.e. the end of the breeding season) and refer to this hereafter as q^*.

To verify that the current model behaves similarly to the C & F model, we evaluated it numerically across a range of realistic parameter values that result in different degrees of asynchrony (quantified as the ratio of d, the average individual life span, to D, the length of the breeding period). We focus specifically upon female d/D because: (1) females are usually the more important sex when considering reproductive phenology; and (2) in the present model males and females may differ both in d and D. We then focus upon the effects on q^* as a function of asynchrony, density, the value of $c(\cdot)$ and the amount of protandry.

MODELS FOR ALTERNATIVE MATING FACTORS

The mating rate function $c(\cdot)$ directly affects the relationship between a given level of asynchrony and q^*, and can be tailored to include alternative mating-factor assumptions. The simplest possible definition of $c(\cdot)$ is to assume that it is constant (Wells *et al.* 1990; Zonneveld & Metz 1991; Zonneveld 1992; Wells *et al.* 1998; but see Zonneveld 1996a). Going beyond this, we assume that the $c(\cdot)$ are power-type functions of a generic, time-dependent mating factor $X(t)$ such that:

$$c(t) = w(X(t))^y \qquad \text{eqn 6}$$

where w and y are fitted parameters to which no direct, biological interpretations are ascribed. As the $X(t)$ we consider are all zero at $t = 0$, when y is negative we use instead:

$$c(t) = w(1 + X(t))^{-y} \qquad \text{eqn 7}$$

to avoid division by zero. When $y = 1$ or -1, these functions simplify to linear and inverse linear. We deviate from equations 6 and 7 for the reasons explained below, when considering female lifetime reproductive opportunities. Next, we derive alternative expressions for $X(t)$. The full set of candidate $c(\cdot)$ models, obtained by substituting these expressions into equations 6 and 7, is summarized in Table 1.

Male age

Male age could affect the mating rate in two conceivable ways. Production of the sphragis is probably metabolically expensive, and some empirical evidence suggests that males produce smaller spermatophores (and presumably smaller sphragis) after their first mating (Wiklund 2003). Therefore, males might become less efficient at mating as they age due to the costs of making new mating plugs. Alternatively, in some species (Kemp *et al.* 2006), older males have an experience-based advantage, and the mating rate may increase with average male age. These effects can be explored by deriving the probability distributions of male ages as a function of time. The probability that a male emerged at time $t - a$ and is still alive at time t is:

$$P(a, t) = g(t - a, \theta_m) \text{Exp}(-\alpha_m a) \qquad \text{eqn 8}$$

where $g(t - a, \theta_m)$ is given by equation 2. The probability distribution of male ages at time t is obtained by normalizing equation 8 (Zonneveld 1992), such that:

Table 1. Candidate mating factor [i.e. $c(\cdot)$] submodels. All models, except model 1 (constant), are based on time-dependent variables that might reasonably be expected to affect mating behaviour. The parameters w and y are estimated by model fit to the data, and are not given direct biological interpretations

Model no.	Model name	Functional form	No. fitted parameters
1	Constant	$c = w$	1
2	Male age	$c(t, w) = w\bar{a}_m(t)$	1
3	Power male age	$c(t, w, y) = w(\bar{a}_m(t))^y$	2
4	Inv. male age	$c(t, w) = w/(1 + \bar{a}_m(t))$	1
5	Inv. power male age	$c(t, w, y) = w/(1 + \bar{a}_m(t))^y$	2
6	Male size	$c(t, w) = wb(t)$	1
7	Power male size	$c(t, w, y) = w(b(t))^y$	2
8	Inv. male density	$c(t, w) = w/(1 + M(t))$	1
9	Inv. power male density	$c(t, w, y) = w/(1 + M(t))^y$	2
10	Female lifetime reprod. opportunities	$c(t, w, y) = w/(1 + yh(t))$	2

$$A_m(a, t) = \frac{g(t - a, \theta_m)\mathrm{Exp}(-\alpha_m a)}{\int_0^t g(t - z, \theta_m)\mathrm{Exp}(-\alpha_m z)dz} \qquad \text{eqn 9}$$

The mean age of males at any time during the breeding season is then:

$$\bar{a}_m(t) = \int_0^t a\, A_m(a, t)da. \qquad \text{eqn 10}$$

Male size

Another possibility is that larger males have an advantage over smaller males in gaining access to females. Assuming exponential growth, Zonneveld (1996a) explored the trade-off between large size and early emergence in butterfly populations. To avoid having to estimate an exponential growth rate separately, we instead assume linear growth, which allows a definition of $c(\cdot)$ where the growth rate can be absorbed into the fitted parameters. We use the expected size difference between males emerging at time t and those emerging at $t = 0$ as an index of male size; thus, assuming constant, linear growth, the total duration of the pre-emergence developmental period does not matter. The expected male size difference on day t of the breeding season is $s(t) = \gamma t$, where γ is the growth rate. The average size difference at any point in time can be written in terms of male age:

$$\bar{s}(t) = \int_0^t \gamma(t - a)A(a, t)da = \gamma b(t) \qquad \text{eqn 11}$$

where $b(t) = t - \bar{a}_m(t)$. Substituting the right-hand side of equation 11 into equation 6, γ can be absorbed into the fitted parameter(s).

Male density

Kokko & Rankin (2006) have argued strongly for the study of density-dependent effects in mating systems. Such effects include males interfering with each other at higher densities and females increasing their search activities and/or decreasing their selectivity at low male densities. As male density, $M(t)$, is given by the solution to equation 1a, no new expression need be defined here.

Female lifetime reproductive opportunities

Females may adjust their behaviour based on the number of expected lifetime reproductive opportunities (Kokko & Mappes 2005). In other words, a female should be selective only when she can expect many future encounters with males. Here, 'choice' may include subtle behaviours such as females increasing their apparency to males or females increasing their own mate-searching efforts actively. An unmated female alive on day t of the breeding season can expect:

$$l(t) = \phi\int_t^{\infty} \mathrm{Exp}(-\alpha_f(z - t))M(z)dz \qquad \text{eqn 12}$$

encounters with males in her remaining life, for constant encounter rate ϕ. It is useful to conceptualize the mating rate function as the product of two components: the mate encounter rate and the probability of mating given encounter (Wells et al. 1990, 1998). Although in practice it is often impossible to separate the two, we use this distinction to allow the probability of acceptance to depend on a female's expected future opportunities. Defining the integral term in equation 12 as $h(t)$ and η as a parameter that scales $l(t)$ independent of the encounter rate, this probability is:

$$P(\text{mating} \mid \text{encounter}) = \frac{1}{1 + \eta\phi h(t)}. \qquad \text{eqn 13}$$

Multiplying equaton 13 by ϕ then yields a functional form for $c(\cdot)$. Making the substitutions $w = \phi$ and $y = \eta\phi$, we can write $c(\cdot)$ in terms of the fitted parameters:

$$c(t, w, y) = \frac{w}{1 + yh(t)}. \qquad \text{eqn 14}$$

Note that because equation 14 includes explicitly a probability (equation 13), which must remain between zero and 1, we have deviated from the forms of equations 6 and 7.

STUDY SPECIES AND DATA SETS

Both butterfly species analysed here, *P. clodius* (Auckland, Debinski & Clark 2004) and *P. smintheus* (Roland, Keyghobadi & Fownes 2000; Matter et al. 2003), are univoltine, have discrete, non-overlapping generations and, due to the sphragis, have monandrous females.

P. clodius was studied in one large meadow in Grand Teton National Park in 1998, 1999 and 2000 (study design in Auckland et al. 2004). Mark–recapture studies were performed to estimate population parameters and to explore the movement of butterflies within the meadow. Sampling continued each year until fewer than five individuals per transect were counted. Only in 2000 were females scored for mating status.

P. smintheus was surveyed in a system of 21 subalpine meadows located in Alberta, Canada (study design in Roland et al. 2000 and

Table 2. Parameter estimates and negative log likelihoods (NLL) for *Parnassius clodius* (PC) and *P. smintheus* (PS) abundance curves. Plain entries are estimated from model fit to the male and female count data. Entries with ± are calculated from the parameterized model, while those with § are mark–recapture estimates taken from Auckland *et al.* (2004). DPE: day of peak emergence. These parameters were used to generate the fitted abundance curves of Fig. 2

		λ	μ	Start date (day 0)	Death rate	Pop. size	Fem. D (days)	Fem. *d/D*	DPE	Days Prot.	NLL
PC	♀	0·46	5·50	14 June 2000	0·56§	113±	28±	0·06±	11·88±	7·22±	−238·90
	♂	1·33	6·18	14 June 2000	0·20§	124±			4·66±		−1177·93
PS	♀	0·26	5·19	18 July 1996	0·33	121±	47±	0·08±	21·61±	2·29±	−136·12
	♂	0·33	6·39	16 July 1996	0·29	720±			19·32±		−2559·74

Matter *et al.* 2004) during 1995–97 and 2001–05. Not all meadows were surveyed in all years. In each year, a meadow was visited three to five times from mid-July to mid-September. Mark–recapture surveys were performed until 75% of all individuals had been recaptured. For our analyses, data were pooled among meadows, within each year.

PARAMETER ESTIMATION AND MODEL COMPARISON

We employ a two-stage strategy to fit the full model (equations 1a, b and 3, with the alternative forms of $c(\cdot)$ shown in Table 1), for which sufficient data (> 10 censuses in a single season) were available for *P. clodius* in 2000 and for *P. smintheus* in 1996 only. The remaining 7 years of *P. smintheus* data are used to provide support for our major results (see supplementary analyses below). For both stages, we assume Poisson distributed observation error and therefore use Poisson likelihood functions (Zonneveld 1991). Appendix SI (see Supplementary material) describes technical details of parameter estimation and model comparison, while here we summarize briefly the general strategy. In the first stage, sex-specific emergence parameters and death rates are estimated by fitting the solutions of equations 1a and b to count data of the number of males and total females, respectively, at census points across the breeding season. A number of quantities that detail population phenology can then be calculated, such as female *d/D*, day of peak emergence (DPE) and amount of protandry (Table 2).

Given the parameters estimated in stage 1, we estimate for each species the parameter(s) of each $c(\cdot)$ candidate model by inserting it into equation 3 and fitting the solution to counts of unmated females across the breeding season. For each parameterized $c(\cdot)$ model, we then calculate q^* and an Akaike's information criterion (AIC)-based ranking (Burnham & Anderson 2002), thus allowing a comparison of the effects of different mating factor assumptions, and an indication of which assumptions are best supported by the data. Finally, we calculate an Akaike-weighted average q^* across all submodels (Burnham & Anderson 2002).

SUPPLEMENTARY ANALYSES

We used the 7 additional years of *P. smintheus* data to test for several key patterns related to reproductive asynchrony. The first was the proportion of observed females that were unmated during each breeding season. Secondly, we pooled data across years and used logistic regression to model, based on date, the probability that: (1) an individual observed is male (a measure of protandry); and (2) observed females are unmated. To accommodate unequal sampling effort among surveys, we consider only within-survey proportions of unmated females for analysis. Multiple censuses at a single site within each year were identified as repeated measures and therefore

were neither pooled nor treated as independent for analysis (Diggle, Lang & Zeger 1994). We included only sites for which data were collected on at least three census dates within a single year. In addition, to calculate proportions, only dates where at least five individuals were observed across all years were included. These analyses were performed using the GENMOD procedure in SAS version 9·1. Results were then compared, within species, to those of the full analyses.

Results

The new reproductive timing model behaves similarly to the individual-based model of C & F. The cumulative number of mated females reaches an asymptote late in the flight period that, unless the average value of the mating function $c(\cdot)$ is high (> 1), will be below the total number of females (Fig. 1a). The proportion of females that ultimately do not mate increases in an accelerating fashion as population density decreases (Fig. 1b; compare with C & F, Fig. 3c) and as $c(\cdot)$ decreases. The severity of these effects tends to increase with increasing asynchrony (as measured by female *d/D*), but is clearly modulated by $c(\cdot)$ (Fig. 1c). The behaviour of the new model differs slightly in two respects from the C & F model. The first is that q^* first decreases and then increases as protandry is increased (Fig. 1d), indicating that an optimal amount of protandry exists under this model, a result consistent with Zonneveld's analyses. In the C & F model, increasing protandry always increased q^*. However, this optimal amount of protandry decreases with increasing asynchrony (Fig. 1d). The second difference is that q^* declines more slowly to zero as population density increases under the new model (Fig. 1b) than in the C & F model. These discrepancies arise from the structural differences between models, including the present model's more realistic assumptions about individual life spans and mating biology.

The basic reproductive timing model for the male and total female abundance curves provides an adequate fit to the field data (Table 2 and Fig. 2). Mark–recapture estimates for the male and female death rates for *P. clodius* were taken from Auckland *et al.* (2004), and thus only three parameters were fitted for each abundance curve (Table 2, Fig. 2a). As a published, within-habitat death rate estimate for the 1996 *P. smintheus* data was unrealistically low (0·01, implying an average individual life span of ~100 days, Matter *et al.* 2004), we chose to estimate this death rate from the count data, along with the other three parameters (Table 2). Calculated
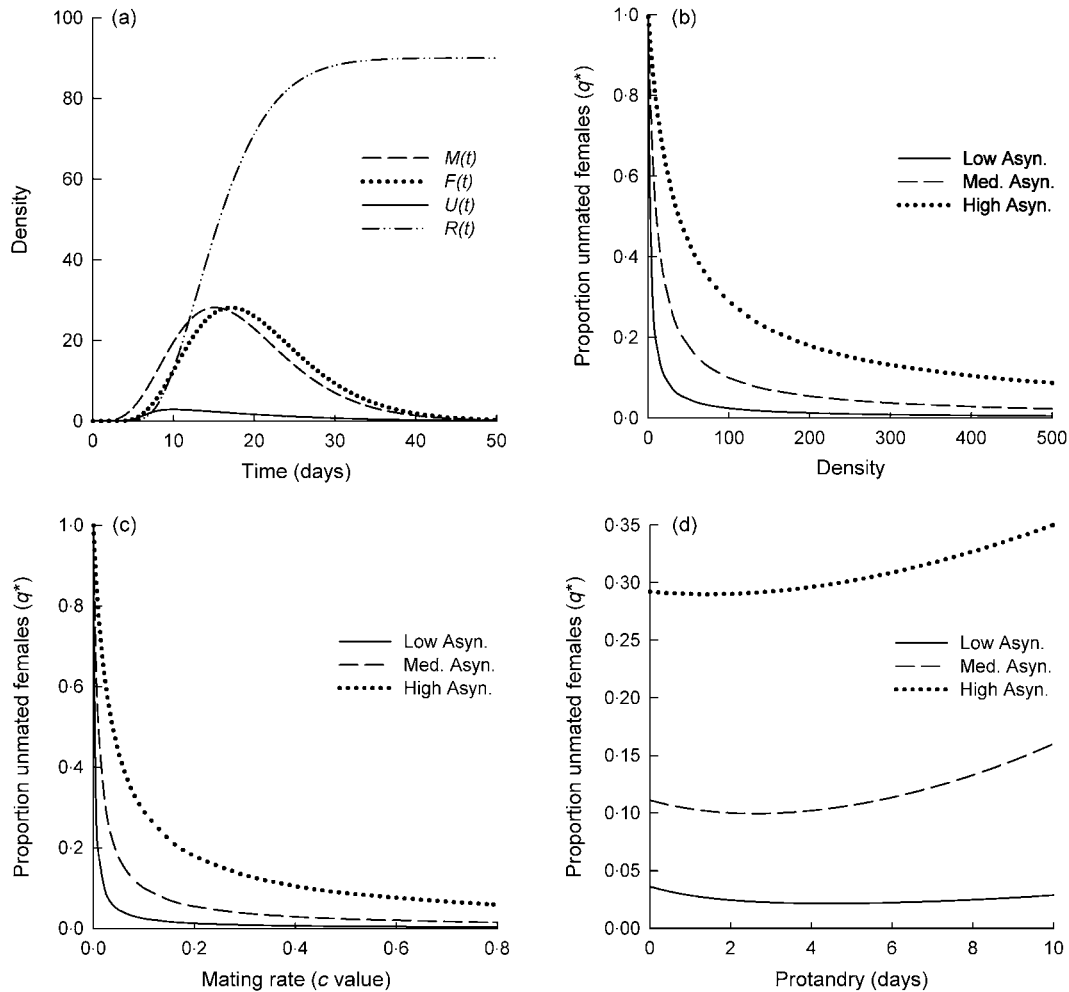
Fig. 1. Behaviour of the reproductive timing model. (a) The instantaneous densities of males [$M(t)$], females [$F(t)$] and unmated females [$U(t)$] and the cumulative density of mated females [$R(t)$] across a breeding season. The remaining panels show dependence of q^* at three different levels of asynchrony (d/D is 0·18, 0·12 and 0·06 for parameter sets 'low asyn.', 'medium asyn.' and 'high asyn.', respectively), on density (b), the (constant) value of $c(\cdot)$ (c), and the amount of protandry (d). The parameters used were similar to those estimated from the data, and the most severe level of asynchrony considered here (set C, $d/D = 0·06$) corresponds with that seen in both *Parnassius* populations (see Table 2). For all results $\mu_m = \mu_f = 5$. For 'low asyn.', $\lambda_m = \lambda_f = 0·6$ and $\alpha_m = \alpha_f = 0·1$. For 'medium asyn.', $\lambda_m = \lambda_f = 0·37$ and $\alpha_m = \alpha_f = 0·2$. For 'high asyn.', $\lambda_m = \lambda_f = 0·2$ and $\alpha_m = \alpha_f = 0·3$. Unless manipulated directly (i.e. on the *x*-axis of the panel), $M_0 = F_0 = 100$, $c = 0·1$ and 2 days of protandry are used. Panel (a) uses the 'medium asyn.' parameter set.

male and female population sizes are very similar for *P. clodius*, while for *P. smintheus* they are skewed heavily towards males. Based on these fitted curves both populations are protandrous, although *P. clodius* is more so, and both populations are quite asynchronous (Table 2). In fact, d/D for both species is high compared to other univoltine butterfly species (see Table A1 in C & F).

Model comparison indicated support for non-constant mating functions across the flight period for both species (Table 3). However, the two species showed contrasting patterns in the time–course of the proportion of unmated females (Fig. 3). For *P. clodius*, the proportion of unmated females was higher towards the beginning of the season than at the end (Fig. 3a); the opposite pattern was apparent for *P. smintheus* (Fig. 3b). As such, mating rate functions that increased sharply throughout the season were supported for *P. clodius* (Fig. 4a). For *P. clodius*,

the one-parameter male age model was best supported, with the one-parameter inverse male density model ranking a distant second (Table 3, Fig. 4a). In contrast, data for *P. smintheus* suggest that the mating rate function should decrease slightly throughout the main part of the flight season (Fig. 4b). Thus, the male density model ranked first, followed very closely by the one-parameter inverse male age model (Table 3, Fig. 4b).

Overall, q^* was quite high for both species, suggesting that reproductive asynchrony could be important in the dynamics of each. Across all mating factor submodels for *P. clodius*, q^* ranged from 0·113 to 0·186, while for the best-supported mating factor model it was 0·131 (Table 3). For *P. smintheus*, q^* ranged from 0·065 to 0·111, with the best-supported model yielding 0·081 (Table 3). The Akaike-weighted multimodel average q^* was 0·138 for *P. clodius* and 0·088 for *P. smintheus* (Table 3).

Table 3. Model comparison results and estimated mating system submodel parameters for *Parnassius clodius* (PC) and *P. smintheus* (PS). Model numbers are as in Table 1 and NLL is the negative log likelihood. For PC, mating factor model 2, male age, performs best [Akaike's information criterion (AIC) diff. of 0, highest Akaike weight] while for PS, model 8, inverse male density, is best supported. The Akaike weights indicate the weight of evidence in favour of each model

	Model	Parameter estimates	NLL	AIC diff.	Akaike weights	Model ranking	q^*
PC	1	$w = 0{\cdot}155$	16·18	5·81	0·025	8	0·153
	2	$w = 0{\cdot}027$	13·27	0	0·462	1	0·131
	3	$w = 0{\cdot}022, y = 1{\cdot}123$	13·24	2·7	0·12	3	0·131
	4	$w = 1{\cdot}126$	21·95	17·37	0	9	0·186
	6	$w = 0{\cdot}03$	15·24	3·95	0·064	6	0·151
	7	$w = 1{\cdot}51 \times 10^{-7}, y = 8{\cdot}404$	13·48	3·18	0·094	4	0·167
	8	$w = 4{\cdot}714$	14·54	2·55	0·129	2	0·113
	9	$w = 3{\cdot}367, y = 0{\cdot}903$	14·52	5·27	0·033	7	0·115
	10	$w = 1{\cdot}846, y = 0{\cdot}263$	13·74	3·7	0·073	5	0·185
PS	1	$w = 0{\cdot}061$	13·91	0·96	0·18	3	0·088
	2	$w = 0{\cdot}024$	17·12	3·45	0·052	7	0·076
	4	$w = 0{\cdot}23$	12·83	0·12	0·273	2	0·102
	5	$w = 0{\cdot}496, y = 1{\cdot}568$	12·67	3·31	0·055	6	0·111
	6	$w = 0{\cdot}004$	16·82	3·21	0·058	4	0·077
	8	$w = 3{\cdot}876$	12·67	0	0·29	1	0·081
	9	$w = 2{\cdot}8, y = 0{\cdot}923$	12·66	3·3	0·056	5	0·081
	10	$w = 0{\cdot}093, y = 0{\cdot}002$	13·76	4·15	0·036	8	0·065

For PC, AIC_c was used to calculate the AIC differences because there was no indication of overdispersion in the data. For PS, the estimated value of the variance inflation factor v (2·58) indicated some overdispersion; therefore $QAIC_c$ was used to calculate the AIC differences. See Appendix SI in Supplementary material for details. Models missing from the candidate set for each species (five for PC, three and seven for PS) are two-parameter functions for which the qualitative pattern of the function was opposite the pattern in the data (e.g. the function increases with time while the data decrease), and thus the estimated value of the exponent y approached zero. In this case, these models effectively reduce to model 1 (constant) and are therefore omitted from the comparison.
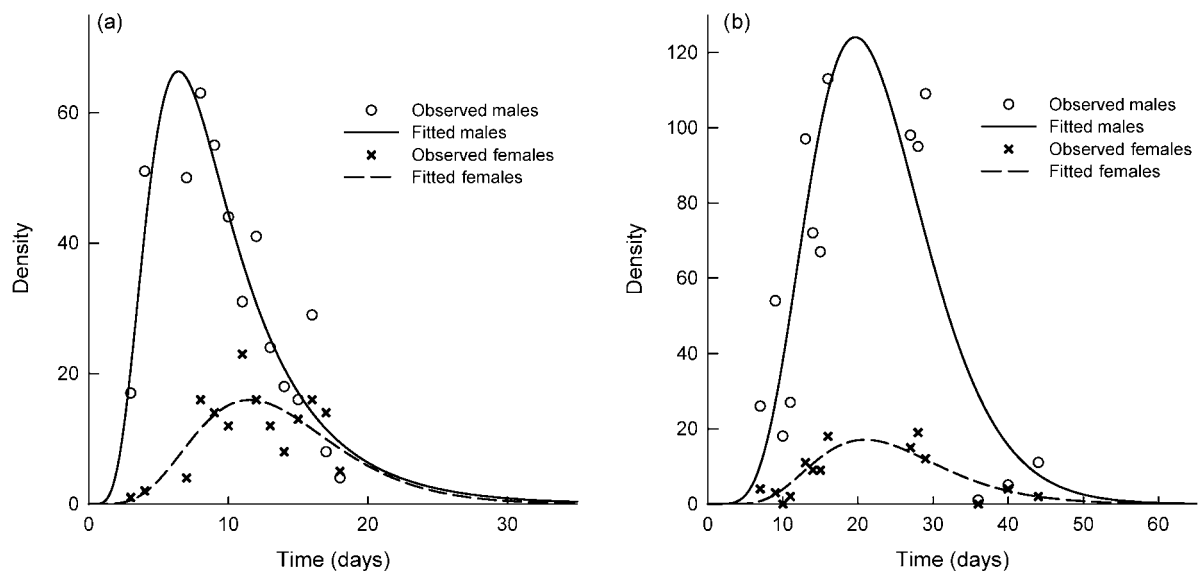

Fig. 2. The fitted density curves for *Parnassius clodius* (a) and *P. smintheus* (b). The general reproductive timing model provides a good description of the data for both species. Estimated parameters and several relevant derived quantities are given in are given in Table 1.

Full analysis of the *P. smintheus* 1996 data suggested that this species is both asynchronous and protandrous (Table 2), that there is an increase in the proportion of unmated females towards the end of the season (Fig. 3b) and that a considerable proportion of the female population dies unmated. For all other years of *P. smintheus* data combined (1995, 1997, 2001–

05), our logistic regression analyses indicated that males tend to be protandrous ($P < 0{\cdot}0001$, Fig. 5a) and that females are more likely to be unmated as the season progresses ($P < 0{\cdot}05$, Fig. 5b). Across the additional 7 years of *P. smintheus* data, the (unweighted) average proportion of unmated females observed was 0·144, ranging from a low of 0·052 in 1995 to a
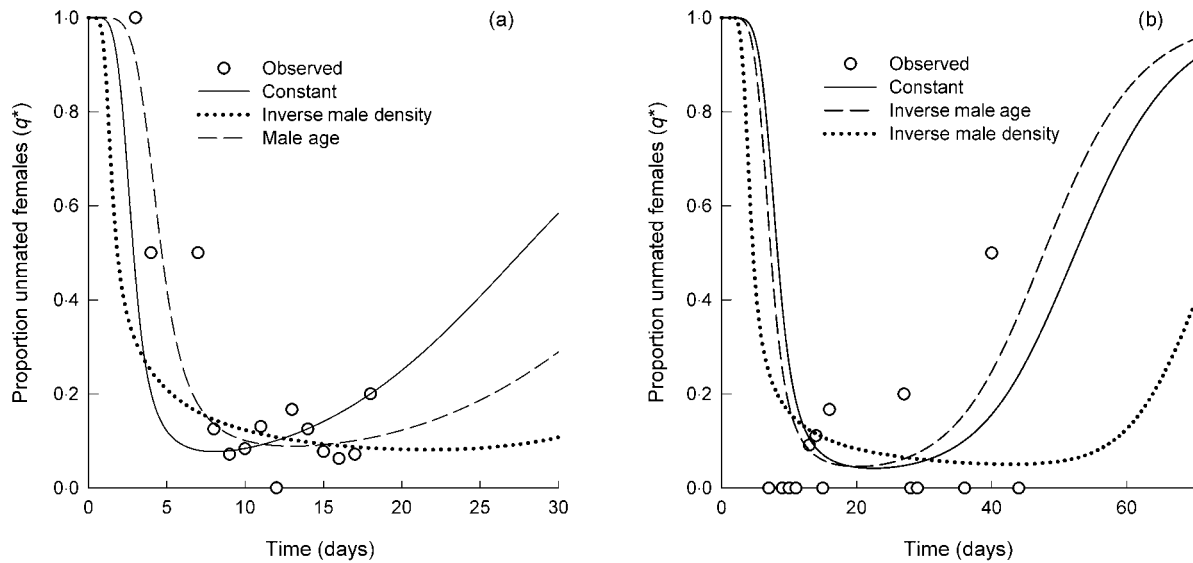
Fig. 3. The instantaneous proportion of unmated females [$U(t)/F(t)$] across the breeding season under three different parameterized mating function submodels. For *Parnassius clodius* (a), average male age is the best-fitting model, followed by inverse male density. For *P. smintheus* (b), inverse male density is best, followed closely by inverse male age. The best-fitting constant mating rate models are shown for reference for both species. The fitted submodels are plotted beyond the range of the data to emphasize that the largest differences among them occur at the extremes of the flight period.
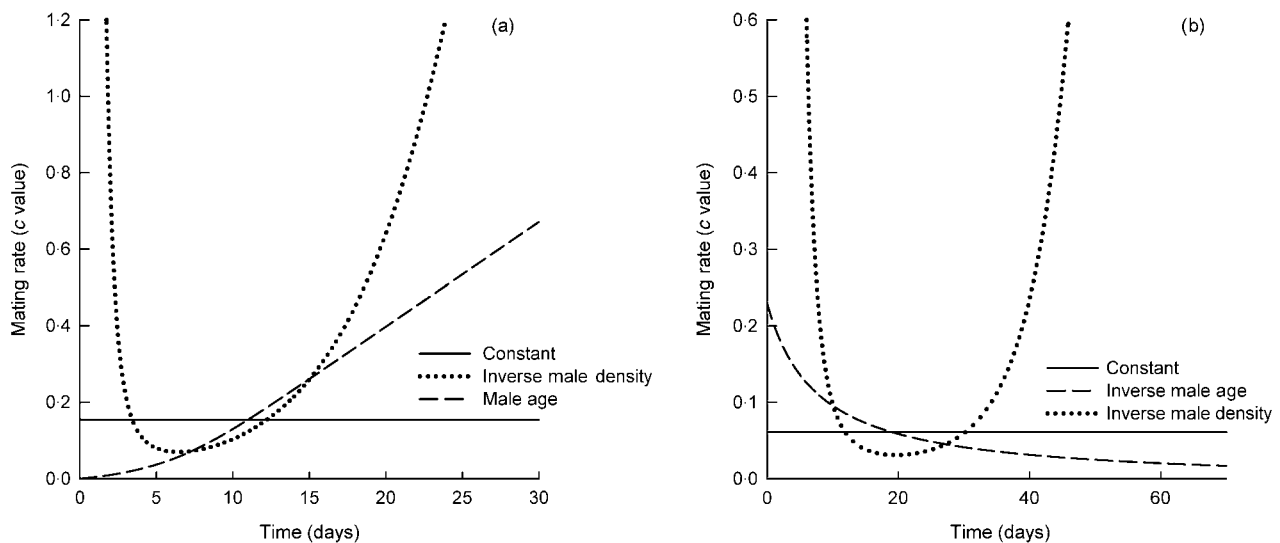
Fig. 4. The shapes of the best two mating factor functions are shown, for reference, with the best-fitting constant mating factor model for *Parnassius clodius* (a) and *P. smintheus* (b). Here the differences among the shapes of these alternative $c(\cdot)$ functions early and late in the season become apparent.

high of 0·237 in 2002. These results agree qualitatively with those from the detailed analysis of 1996 data, and suggest that protandry, an increase in female matelessness with time and a relatively large proportion of unmated females are general features of the reproductive phenology of *P. smintheus*.

Discussion

Reproductive asynchrony is widespread in nature (Calabrese & Fagan 2004), but its effects on population growth potential have received little attention. The analyses of C & F suggested that many species of butterflies, among other taxa, exhibit

levels of asynchrony that could cause an Allee effect in low-density populations. Here, we have quantified this lost reproductive potential by developing a general framework for exploring reproductive phenology that accommodates alternative mating-factor assumptions and is connected easily to data. This approach facilitates new insights into how asynchrony affects female mating success in low-density natural butterfly populations and could be used easily for other taxa with asynchronous mating dynamics.

Our main result is that reproductive asynchrony can lead to a substantial proportion of females dying unmated in natural, low-density populations, even in the presence of mating factors
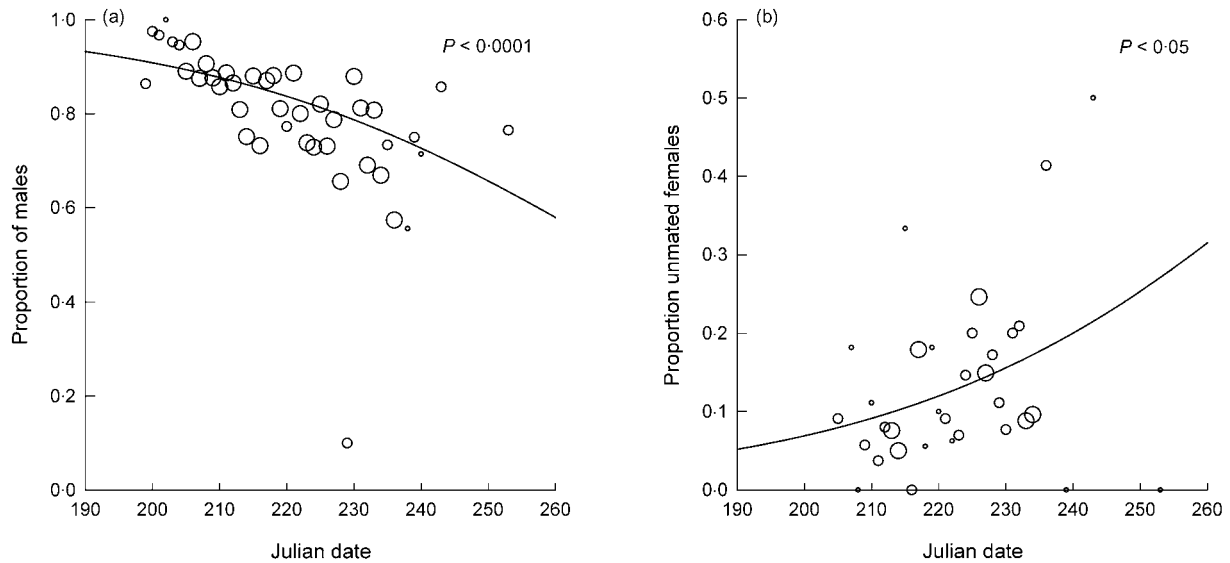
Fig. 5. The probability as the season progresses that an individual observed is male (a) and female observed is unmated (b). Proportions are calculated by date pooled across survey years (1995, 1997, 2001–05). The average number of observations per date was 150 for the analysis of male proportions and 30 for the analysis of female mating success. Circle size corresponds to the number of observations used to calculate each proportion (5–20 for small circles, 21–50 for medium, > 50 for large). The *P*-values and predicted proportions are based on logistic regression.

that compensate for the negative effects of asynchrony. The total (season-long) proportion of unmated females in these *Parnassius* populations was large enough (q^*, ranging between 0·065 and 0·186, Table 3) to have a sizeable effect on population dynamics. Assuming simple geometric population growth, these proportions imply that average per-capita, per-year population growth rates resulting from mated females must exceed 6·9% at the low end and 22·8% at the high end (see C & F, equation 2) to avoid collapse due to an Allee effect. Furthermore, the loss of reproductive potential due to asynchrony is density-dependent and increases sharply as population densities decline (Fig. 1b).

The mating factor submodels in our analyses explore the effects of alternative mating strategies on female mateless-ness. Incorporation of these features modulates, but does not eliminate, the negative effects of asynchrony in these *Parnassius* populations. This suggests that these species may not compensate behaviourally for the loss of reproductive potential in low-density asynchronous populations. The analyses also shed light on important features of the mating biology of these species. While we urge caution in this type of interpretation based on the present, rather limited, data we will venture some plausible explanations for the observed patterns. The decreasing proportion of unmated females over time for *P. clodius* (Fig. 3a) suggests a mating function that increases across the season, the most parsimonious of which was average male age. Possible biological explanations include males gaining experience as they age and/or older males having a competitive advantage. Similar effects are seen in other butterfly species (Kemp *et al.* 2006), but it is not clear if they are a factor in *P. clodius*. Alternatively, some unidentified time-correlated factor may be at work, but there were no clear patterns with such possibilities as temperature or weather

(results not shown). Additionally, inverse male density was also relatively well supported, suggesting that female receptivity or search effort increases at low male density and/or males may interfere with each other at high density. The latter phenomenon has been observed in some *Parnassius* populations (Scott 1974; S. F. Matter, personal observation).

Both sets of analyses on *P. smintheus* point to it being protandrous, and showing an increasing proportion of unmated females with time. Thus, at least qualitatively, reproductive phenology of *P. smintheus* is consistent across years and meadows, suggesting that our detailed analyses of the 1996 data are robust. The best descriptor of this increasing pattern was, narrowly, inverse male density, while inverse male age ranked a close second (differing by only 0·12 QAICc units). Male density may be important for the reasons outlined above, while potential energetic and time costs of producing the sphragis could account for the decrease in realized mating efficiency with increasing male age. Male density, which ranked high for both species despite qualitative differences in the time–course of unmated females, may be a key factor to focus on when studying the mating biology of *Parnassius* butterflies.

Our analyses also highlight the data required to understand more clearly reproductive dynamics in asynchronous populations. Complete coverage of the flight period is important to select unambiguously among alternative $c(\cdot)$ submodels, as the biggest differences among these models occur at the beginning and end of the season (Figs 3 and 4). Furthermore, the qualitative difference between the two species in the time–course of unmated females may result from unequal coverage of the extremes of the flight period between the two studies. Specifically, observations on *P. clodius* from three field seasons (1998–2000) indicate that unmated females become proportionately more common very late in the season,

after formal sampling had stopped (J. N. Auckland, personal observation). This suggests that the two species may be more similar than our analysis implies. It is important to note, however, that our $q*$ results are robust to a lack of sampling coverage at the season extremes simply because the vast majority of $q*$ accumulates across the middle of the season, when the bulk of the population is active and sampling coverage is good. Another sampling issue is the importance of accounting for males and females separately when mating processes and Allee effects are considered (Boukal & Berec 2002; Calabrese & Fagan 2004), and we suggest that field studies take note of this advice. Although *Parnassius* and other sphragis-bearing butterflies are studied regularly, data on unmated females are almost never reported. As we have shown, such information can be used to explore mating dynamics across the breeding season at a detailed, mechanistic level. Another potential issue is that detectability can differ between sexes in butterfly populations, and may account for the apparently male-biased sex ratio we observed for *P. smintheus* (Roland *et al.* 2000; Matter & Roland 2002; Matter *et al.* 2003) and the high female death rate estimated for *P. clodius* (Scott 1973; Matsumoto 1985; Auckland *et al.* 2004). However, when detectability is allowed to differ between the sexes (females being less detectable), a 1 : 1 sex ratio is assumed, and female death rates are assumed equal to male death rates (and therefore lower), our estimates of $q*$ are hardly affected, suggesting our results are robust to these potential biases (results not shown).

Although our $q*$ results appear contrary to the conventional wisdom on female reproductive success, the literature does contain hints that female matelessness may be considerably more widespread than realized. Following Kokko & Mappes (2005), Appendix SII (see Supplementary material) gives detailed examples of asynchronous Lepidoptera species exhibiting between 4% and 18% of observed females unmated. It is difficult to establish if such percentages are truly representative, because the proportion of unmated females in natural populations is reported rarely. While many species will undoubtedly have high average female reproductive success, particularly in high-density conditions, the dearth of observations of unmated females in Lepidopteran populations may reflect an observability bias. The effects of asynchrony on $q*$ are expected to be most pronounced at low density. If researchers choose their study populations such that densities are high enough that large samples sizes can be collected consistently, the existence of unmated females in low-density populations may go unnoticed. Additionally, the females most likely to die unmated, all else being equal, are those with the shortest life spans: those most difficult to observe.

Taken together, the results of the present study, the examples of Appendix SII (see Supplementary material), and the review undertaken by C & F (demonstrating widespread asynchrony), suggest clearly that the effects of asynchrony on female reproductive success warrant further study across a range of taxa. The framework introduced here provides a general, quantitative and data-friendly description of mating success in asynchronous populations and could help to unify such future work. For now, our quantitative approach facilitates new insights into the interplay between phenology and reproductive success in natural butterfly populations, and confirms previous suggestions that asynchrony substantially reduces a population's growth potential.

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Supplementary material

The following supplementary material is available for this article.

Appendix SI. Parameter estimation and model comparison methods.

Appendix S2. A brief review of female reproductive failure in the Lepidoptera.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2656.2008.01385.x.
(This link will take you to the article abstract).

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