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Evolutionary constraints on polyembryony in parasitic wasps: a simulation model

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Polyembryony involves the production of several genetically identical progeny from a single egg through clonal division. Although polyembryonic development allows highly efficient reproduction, especially in some parasitoid wasps, it is far less common than monoembryony (development of one embryo per egg). To understand what might constrain the evolutionary success of polyembryony in parasitoids, we developed Monte Carlo models that simulate the competition between polyembryonic females and their monoembryonic counterparts. We investigated which simulated life-history traits of the females allow the monoembryonic mode of development to succeed. Published empirical studies were surveyed to explore whether these traits indeed differ between polyembryonic parasitoids and related monoembryonic species. The simulations predict an advantage to monoembryony in parasitoids whose reproduction is limited by host availability rather than by egg supply, and that parasitize small-bodied hosts. Comparative data on the parasitoid families Encyrtidae and (to a lesser extent) Braconidae, but not the data from Platygastridae, circumstantially support these predictions. The model also predicts monoembryony to outcompete polyembryony when: 1) hosts vary considerably in quality, 2) polyembryonic development carries high physiological costs, and 3) monoembryonic females make optimal clutch size decisions upon attacking hosts. These multiple constraints may account for the rarity of polyembryony among parasitoid species.

Keywords: polyembryony, parasitoids, Monte Carlo simulation, evolutionary constraints, literature survey

Introduction

Obligate polyembryony is a mode of development that involves asexual proliferation of sexually produced embryos to form genetically identical sibling clones. It resembles monozygotic twinning (Gleeson et al. 1994), but usually involves more than two individuals per clone. Polyembryony has evolved independently in several plant and animal taxa (Craig et al. 1995, 1997). It is particularly intriguing in parasitoid wasps, insects whose larvae consume a single arthropod host during their development.

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In some polyembryonic parasitoid species, thousands of individuals develop from each egg within the host's body (Strand 2003). Polyembryony in parasitoids is also sometimes associated with a larval soldier caste and with a kin-recognition mechanism, thus showing some features of social organization (Giron et al. 2004). The extreme potential of polyembryonic parasitoids to reproduce clonally renders them attractive models for evolutionary studies of this developmental mode (Ode et al. 2018). Here, we combine modeling with empirical life-history information to ask why monoembryony (i.e. sexual reproduction without clonal proliferation) predominates over polyembryony in parasitoid species.

Three main hypotheses for the evolutionary advantages of polyembryony in parasitoids have been considered. First, polyembryonic development may provide a way for females to overcome egg limitation. In other words, the cloning of embryos may allow a high reproductive output even in parasitoid species in which females have more oviposition opportunities than eggs (Segoli et al. 2010b). Moreover, small host size at the time of parasitism may constrain the number of wasp eggs laid. The cloning mechanism allows polyembryonic species to increase their broods later on, as their hosts develop and grow. Second, it may alleviate conflict and aggression (which often leads to mortality) between larvae that develop within the same host, as all of the developing clone-mates have identical genotypes (Godfray 1994). A third hypothesis is inspired by the observation that all of the polyembryonic parasitoid species are koinobionts, which means that their hosts continue growing while parasitized. The hosts are typically attacked as eggs or young larvae, and the parasitoids' progeny emerge when the host reaches an advanced larval stage. This feature implies longer developmental durations (compared to idiobiont species that arrest their host's development), with immature parasitoids sometimes even overwintering within the hosts. Consequently, parasitoid females may not be able to accurately predict the future quality of the host for their developing offspring at the time of oviposition. It has been suggested that the embryonic proliferation allows efficient adjustment of brood size to the carrying capacity of the host when that final carrying capacity cannot be foreseen by the parents (Craig et al. 1995, 1997). This would provide polyembryonic females with a selective advantage over monoembryonic ones, in which the number of eggs laid into a host might exceed its eventual carrying capacity resulting in parasitoid mortality.

In spite of its potentially large advantages, polyembryonic development is the exception rather than the rule among parasitoid wasps. Polyembryony has evolved in only four out of about 48 families of parasitoids (Strand 2003). Within the Dryinidae, polyembryony has been described in the species *Aphelopus thelliae* only. Among the Encyrtidae, it has been described in 18 species of the genera *Ageniaspis*, *Copidosoma* and *Coelopencyrtus*. The developmental mode of the hundreds of remaining species in the genera *Aphelopus* (Dryinidae), *Ageniaspis*, *Copidosoma* and *Coelopencyrtus* (Encyrtidae) is still unknown but most are suspected to be polyembryonic based on their life-histories. Polyembryonic development has also been found in four species of the genus *Macrocentrus* (Braconidae) and in six species of *Platygaster* (Platygastridae). Nineteen species of *Macrocentrus* and nine species of *Platygaster*, however, are known to develop monoembryonically.

What are the selective constraints that prevent polyembryony from evolving in additional lineages of parasitoids? Possibly, the putative selective advantages of polyembryonic development do not apply to all parasitoid species. For example, if overcoming egg limitation is a major evolutionary driver of polyembryony, then it may not evolve in species that are more time- than egg-limited (Segoli et al. 2010b). Similarly, in species where the host's carrying capacity can be reliably predicted by an ovipositing parasitoid, polyembryony would not be selected as a mechanism to optimize clutch size (Craig et al. 1997).

The hypotheses outlined so far focus on possible benefits of clonal development, and predict conditions that minimize these benefits to limit the evolution of polyembryony. A complementary set of hypotheses aims to identify fitness costs that are associated with polyembryony. Conditions that increase these costs would be predicted to restrict polyembryony's evolutionary spread. Craig et al. (1995, 1997) suggested that the main drawback of polyembryony is the loss of genetic variation among clone-mate offspring, which would reduce the potential adaptation of the progeny to variable, unpredictable environmental conditions. On the other hand, as the offspring are sexually produced, they differ genetically from their parents. Thus, they might also have lower fitness than asexually produced individuals in environments that remain stable over time. Hardy (1995) pointed out that polyembryony leads to a loss of within-clone genetic variation only, but might not affect the overall population-level genetic diversity since each mother produces several clones through sexual reproduction. The resulting among-clone genetic diversity could potentially be equivalent to the diversity among individuals in a monoembryonic wasp population. The within-clone genetic identity, however, does potentially reduce parasitoids' ability to cope with variable host phenotypes. This is because, in a host representing a poor developmental environment for a given parasitoid genotype, all progeny having this same genotype will have a lower fitness. It is not known yet whether such a potential disadvantage is sufficient to explain the rarity of polyembryony among parasitoid species. A further possible constraint on the evolution of polyembryony is a fitness penalty that polyembryonic parasitoids may incur during their development, and which we denote as 'proliferation cost'. This is the cost associated with the genetic (Corley et al. 2005), endocrine (Strand et al. 1991) and developmental (Gordon and Strand 2009) processes required for the proliferation mechanism. Potential mechanisms of such cost include a longer period required for embryonic proliferation of parasitoid larvae within starved hosts (Saeki and Crowley 2013), reduced clone sizes in wasps that need to detoxify secondary metabolites during development (Ode et al. 2004), or reductions in individual wasp body size associated with increased clonal proliferation (Saeki et al. 2009, Segoli et al. 2009).

We developed Monte Carlo simulation models to explore several hypotheses potentially explaining the rarity of polyembryony among parasitic wasps. The simulations track the outcomes of the competition between polyembryonic females and their monoembryonic counterparts over several generations. The monoembryonic parasitoids are modeled as gregarious (that is, several offspring, each from a different egg, can successfully develop in one host), to allow comparison between clonal and non-clonal clutches of several individuals. With these models, we tested whether reducing some hypothesized benefits of polyembryonic development, or increasing its putative costs, provide a sufficient selective advantage to monoembryony. More accurately, we decreased the risks of egg limitation and of laying suboptimal clutches for the monoembryonic competitors, so as to diminish the relative advantages of polyembryony. We also enhanced the disadvantages of polyembryonic development by increasing its associated proliferation cost and by simulating variable host environments that might penalize low genetic variation. The simulation approach allowed us to easily model environmental and genetic stochasticity, and to test how these variability components impact the success of the competing strategies. Moreover, we used sensitivity analyses to explore the potential effects of variables that have not been experimentally measured yet. These include the proliferation cost for the polyembryonic females and the risk of laying a larger number of eggs in a host than its carrying capacity for the monoembryonic females. Based on the outcomes of these manipulations, we generate predictions regarding the main evolutionary constraints on polyembryony. Finally, we performed a literature survey to see whether these predictions can be supported in different families of parasitic wasps.

Methods

The modeling approach

To identify under what conditions a polyembryonic reproduction mode can be inferior to monoembryonic reproduction, we used the following modeling framework: starting with a population having the same number of polyembryonic and monoembryonic gregarious individuals, our models simulated the competition process between these two modes of reproduction over several generations, up to the moment one mode of reproduction completely invaded the population. To keep the models as simple as possible, no recombination was considered, hence simulated individuals were modeled as thelytokous parthenogenetic females. Also, the females' egg-maturation process was not implemented in the simulations, thus all eggs in the ovaries are assumed to be ready for oviposition when hosts are encountered (Jervis et al. 2001). Finally, wasps are modeled as koinobionts, and a host's final body size determines its carrying capacity for the developing parasitoid progeny.

The number of mature eggs in the females' ovaries limits their reproductive success in some cases, a constraint known as egg limitation (Heimpel and Rosenheim 1998). In other situations, parasitoid reproduction is more restricted by the number of suitable hosts or by the time available to find them (host/time limitation; Phillips and Kean 2017). To evaluate the factors that potentially constrain the evolution of polyembryony, we developed two different simulation models that illustrate two extreme cases. One assumes egg limitation only, while the other simulates the reproductive behavior of wasps that are exclusively time-limited. The severity of egg versus time limitation changes dynamically over the adult lifespan of parasitoids (Casas et al. 2000). By modeling each limiting factor separately, we were able to compare their consequences for polyembryony.

The egg-limited model

In the egg-limited scenario, and in every generation, each female starts with a finite number of eggs to be laid depending on its mode of reproduction. Polyembryonic and monoembryonic females can potentially lay Ec_p and Ec_m eggs in their life, respectively. To explore the potential role of polyembryony in overcoming egg limitation, Ec_p was initially considered to be far lower than Ec_m (Table 1). We then complemented these simulations with a sensitivity analysis, where the overall potential fecundity of the polyembryonic females (Ec_p) was varied (within the [1–100] interval) to quantify the effect of egg limitation on the model's outputs. At the beginning of each simulation, a genetically determined trait value was drawn from a Normal distribution with mean μ_g and standard deviation SD_g, for all eggs of both types of females. The matching between the trait value of each egg and the type of its host determines the egg's ability to develop in the host and hence defines its fitness. Thus, the trait value assignment process corresponds to heritable variation among wasps, and allows us to assess how this variation can affect the relative fitness of monoembryonic versus polyembryonic females under different environmental conditions. At each generation, for all females and up to the moment all of them have laid all their eggs, hosts were sequentially encountered. The hosts' final sizes (reflecting the number of parasitoids they can sustain) were drawn from another Normal distribution with mean μ_h and standard deviation SD_h. Polyembryonic females lay one egg per host. In each attacked host, the number of developing progeny can potentially equal the final host size (by proliferation) and they all have the same genotype. A proliferation cost P was incorporated in the model to reflect the extra metabolic and biochemical investment required for the polyembryonic proliferation process. This cost is expected to increase with the final host size, because larger hosts require more proliferation. In the simulations, it was modeled as a proportion of developing progeny that die within the host before adult emergence, linearly linked to the host size, and

Table 1. Definition of all parameters of the models developed to simulate egg-limited or time-limited females, separating parameters that are common to both models from those that are specific to each model separately. Values in square brackets denote parameter ranges explored in the sensitivity analyses.

	Parameter	Value(s) used
Parameters common to both models		
μ_g	Average of the distribution of all parasitoid genetically determined trait values in the population	100.0
SD_g	Standard deviation of the distribution of all parasitoid genetically determined trait values in the population	20.0
μ_h	Average size of the hosts attacked	{15.0; 20.0; 23.0; 24.0; 24.5; 25.0; 30.0}
SD_h	Standard deviation of the size of the hosts attacked	5
P	Physiological cost associated with polyembryonic proliferation	0.0125 × host size – 0.075 (slope of the relationship ϵ [0.0–2.0])
μ_m	Average error monoembryonic females make upon encountering a host to decide how many eggs to lay	0.0
SD_m	Standard deviation of the error monoembryonic females make upon encountering a host to decide how many eggs to lay	5.0 ([0–18])
μ_e	Environmental condition (host quality/type) under which the average fitness of the population is maximal	100.0
SD_e	Standard deviation of the environmental condition (host quality/type)	{0.0; 20.0; 40.0; 60.0; 80.0; 100.0}
ω	Width of the environmental niche defining the environmental condition (host quality/type)	1.0
Parameters specific to the egg-limited model		
Ec_p	Initial egg complement of polyembryonic females	5 ([0–100])
Ec_m	Initial egg complement of monoembryonic females	100
Parameters specific to the time-limited model		
S_p	Survival probability at the end of the foraging bout for polyembryonic females	0.2
S_m	Survival probability at the end of the foraging bout for monoembryonic females	0.2
H_p	Number of hosts encountered in a foraging bout by polyembryonic females	7
H_m	Number of hosts encountered in a foraging bout by monoembryonic females	7

bound to remain in the [0.0–1.0] interval (Table 1). Here too, we performed a sensitivity analysis to test how different proliferation cost levels affect the simulation's outcome. For this, the slope of the linear link between the proliferation cost and host size (Table 1) was varied within the [0.0–2.0] interval. Monoembryonic females, on the other hand, lay a number of eggs corresponding to the expected final size of each host encountered and do not pay an egg maturation cost. However, the number of offspring that eventually emerge from a host is often lower than the number of eggs laid by the females. This is because monoembryonic females may make mistakes in estimating the final size of the host encountered while deciding how many eggs to lay in it (Godfray 1994). This mis-estimation error, often observed in koinobiont species (Elzinga et al. 2005, de S Pereira et al. 2017), might lead monoembryonic females to lay non-optimal clutches in attacked hosts. Polyembryonic wasps, on the other hand, have a longer period of developmental interaction with the host (the proliferation phase) that allows them to adjust brood size to host size. The mistake, for the monoembryonic females, was drawn in each case from a Normal distribution with average μ_m (set to 0.0) and standard deviation SD_m. Low mistake values are likely to occur in parasitoids that exert some control over their hosts' final size, by manipulating the feeding rates or the number of juvenile instar on the hosts. Parasitoids that do not manipulate their hosts' development may be prone to higher mistake rates. Clutch sizes laid were calculated as the sum of the host's size and the current mistake value. If the number of eggs laid in a host exceeds its size, only the number of progeny corresponding to the final host size survives and all other progeny (chosen randomly) die. If the clutch is smaller than the carrying capacity of the hosts, all progeny complete their development. Females also lay suboptimal clutches if insufficient eggs remain in their ovaries to match the expected final host size, in which case all remaining eggs are laid. In a further sensitivity analysis, we manipulated the value of SD_m (within the [0–18] interval) and recorded the resulting outcome of the simulated competitions.

The fitness of each adult emerging at the next generation was assumed to depend on its genetically determined trait value and on its developmental environment, defined by the quality/type of the host in which it developed. Host quality/type was considered to be independent of its final size, and its value E was drawn from a Normal distribution with mean μ_e and standard deviation SD_e. μ_e is the host quality/type under which the average fitness of the parasitoid population is maximal.

The fitness of each adult progeny was defined using the following Gaussian equation:

$$F = e^{-\frac{(x-E)^2}{2\omega^2}} \tag{1}$$

where x is the genetically determined trait value of the individual and ω is the standard deviation of the distribution of all possible host quality/types (i.e. the width of the environmental niche) (Haldane 1954, Roff 1997). Without any loss of generality, all computations were done with $\omega = 1.0$ in this work. Parasitoids attain optimal fitness when their trait value is identical to host quality/type, namely when they are perfectly adapted to their host.

Just like in genetic algorithms (Sumida et al. 1990, Ruxton and Beauchamp 2008, Hoffmeister and Wajnberg 2008), the individuals at each generation that contribute to the next were chosen using a roulette wheel selection process (Bäck 1996), in which individuals with the higher fitness have a higher chance of being selected. This random process imitated natural selection, and enabled us to choose the mode of reproduction of all females and the genotype of all of their eggs.

At the beginning of each simulation, a population of 1000 individuals comprising 500 polyembryonic and 500 monoembryonic females was considered. At each generation, individuals were drawn randomly with replacement following the selection process described above until a new population of 1000 individuals was reached. The reproduction-selection process was then repeated until fixation of one of the developmental modes.

The time-limited model

This version of the model was built following the same general principles of reproduction, costs, selection, etc., as the egg-limited model described above. However, in this case, the foraging process of both types of females consisted of a succession of foraging bouts, in which hosts are encountered and attacked. Each generation ceased when all females died.

We also considered the possibility that monoembryonic and polyembryonic females differ in the extent of time and host limitation that they experience and in their host seeking capability. This might be the case, for example, if monoembryonic and polyembryonic species differ in body size. This was modeled by assigning, for each foraging bout, a survival probability (S_m or S_p) and a host encounter rate (H_m or H_p) to the monoembryonic and polyembryonic females (respectively). Lower survival and host encounter rates correspond to a more severe time/host limitation. We initially we set $S_m = S_p$ and $H_m = H_p$, imposing equal limitations on polyembryonic and monoembryonic parasitoids (Table 1). In later simulations, we introduced asymmetries in time limitation and host seeking efficacy between the two modes of development ($S_m = 0.4$, $S_p = 0.2$ and $H_m = 8$, $H_p = 7$). This created stronger time limitations on polyembryonic parasitoids than on the monoembryonic ones, analogous to the more severe egg limitation constraints set for polyembryony in the egg-limited model.

Parameter values manipulated in the simulations

Simulation parameters were manipulated (Table 1) to identify the main factors that limit the spread of polyembryony. This was done by reducing the putative selective benefits of polyembryonic development or by increasing its costs (Table 2). When considering the possible benefit of polyembryony in alleviating egg limitation, we expected that time-limited wasps (which are simulated as having theoretically an infinite egg load) would gain less from polyembryonic development than egg-limited females. We also predicted that monoembryonic wasps are less likely to become egg-limited

Table 2. Summary of the evolutionary hypotheses tested in the simulations and whether these hypotheses were confirmed by the results obtained. Tests 2 and 6 predict opposite effects of host size on the success of polyembryonic development. We used the simulation results to infer which of these opposing effects is stronger.

Hypothesized effect of polyembryony	Expected effect on the fitness of individuals	Test performed in the simulations	Expected simulation outcome	Confirmed by simulation results?
Reduction of the egg limitation constraint	positive	1. Compare egg-limited and time-limited models	lower polyembryony success under time limitation	yes
		2. Manipulate average final host size	lower polyembryony success in smaller hosts	yes, under egg limitation
Optimization of clutch size	positive	3. Vary error of monoembryonic females in predicting final host size	lower polyembryony success when error is reduced	yes
Reduction of within-clone genetic variability	negative	4. Manipulate variability in the environment (host quality/type)	lower polyembryony success when among-host variability increases	yes, in intermediate-sized hosts and under egg limitation
Proliferation cost	negative	5. Vary proliferation cost	lower polyembryony success when proliferation cost is increased	yes
		6. Manipulate average final host size	lower polyembryony success in larger hosts	no

when parasitizing smaller hosts (where optimal clutch sizes are lower) than when laying many eggs into larger hosts. We tested these predictions by running simulations under both egg and time limitation, by varying the final host body sizes, and by varying the EC_p / EC_m ratio in the egg-limited model.

A second hypothesized benefit of polyembryony is that it leads to optimal clutch sizes by adjusting embryonic proliferation to host size, whereas monoembryonic females are prone to mis-estimating their hosts' carrying capacity. To test this possibility, we either incorporated errors made by monoembryonic females when deciding how many eggs to lay in a host, or eliminated this risk of error. We expected the relative success of polyembryony to decrease when monoembryonic females better assess the hosts' carrying capacity.

To study whether the cost of reduced genetic variability can be a barrier to polyembryony, we manipulated the variation in host quality/type (SD_e) in the simulations. This followed the reasoning that low genetic variability should be detrimental mostly in heterogeneous, unpredictable environments. Since all polyembryonic progeny developing in a host have the same genotype, we expected all of them to be optimally adapted to a small number of host qualities/types. Genetic variability within the clutch, as in monoembryonic development, is predicted to improve adaptation to heterogeneous environments comprised of many types of hosts. The genetic variability among clutches, on the other hand, is similar in the monoembryonic and polyembryonic development, and should not cause differences in their adaptation to variable environments. We therefore predicted the success of polyembryony to decrease with increasing levels of variability in host quality/type.

Finally, to evaluate the role of the proliferation cost in limiting polyembryony, we ran the simulations while gradually increasing this cost. Note that the proliferation cost was modeled as a positive linear function of final host size. Thus, larger hosts are predicted to generate both disadvantages for monoembryonic females (by increasing the risk of egg limitation) and to their polyembryonic competitors (by increasing the proliferation cost, Table 2). We tested which of these conflicting effects had a greater influence on the simulation's outcome.

Table 1 lists all parameters of the two simulation models, their meaning and the values used. Table 2 details the hypotheses tested by varying the simulations' parameters and the expected outcomes, and summarizes the results obtained. The simulations were repeated 100 times for each combination of tested parameter values. The proportion of replicates in which the polyembryonic mode of reproduction won was recorded in all sets of simulations.

Literature survey

To test the main predictions of the simulation models described above, we compiled published information on the parasitoid body sizes, clutch sizes, final host sizes and lifetime fecundities of polyembryonic species and their monoembryonic gregarious relatives. Our survey focuses on the families Braconidae, Platygastridae and Encyrtidae, which are relatively well-studied. The biology of most Dryinidae is virtually unknown, and they were thus excluded from the survey. Monoembryonic and polyembryonic species can be compared within the genera *Macrocentrus* (Braconidae) and *Platygaster* (Platygastridae). We found relevant (but often incomplete) life-history information on 13 species of *Macrocentrus* and nine species of *Platygaster*. No monoembryonic species are known within the genera *Aphelopus*, *Ageniaspis*, *Copidosoma* and *Coelopencyrtus* (Encyrtidae). We therefore compared data on 18 polyembryonic species from these genera with monoembryonic encyrtids of different genera that parasitize hosts of the same insect orders. We were not able to correct for phylogenetic distance between the species and genera in our dataset because the evolutionary relationships within and between these parasitoid families are still largely unknown.

Results

The egg-limited model

Figure 1 describes the situations in which the polyembryonic mode of reproduction wins the competition in the egg-limited model with different average final host sizes and under different levels of variation in the quality/type of hosts in which parasitoid progeny developed. When polyembryonic females encounter and attack hosts with large final body sizes, the proliferation mechanism enables them to produce many progeny from a small number of eggs laid, and monoembryonic females then cannot survive the competition over several generations of simulation. On small hosts, however,

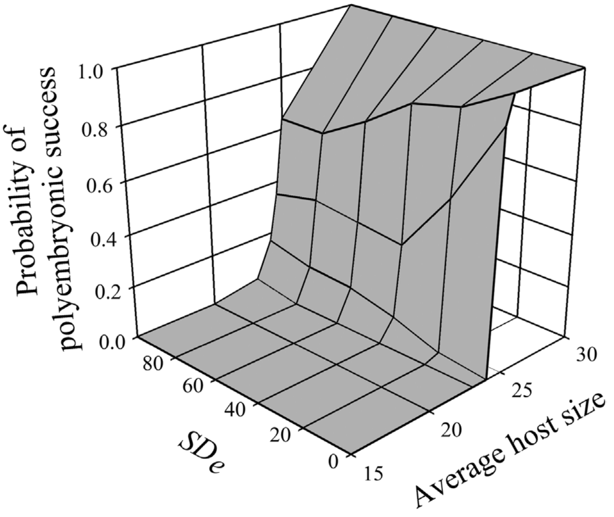

Figure 1. Results of the egg-limited model. The graph shows the proportion, in each case among 100 replicates, of cases in which polyembryonic females win the competition again their monoembryonic counterparts with different average sizes of the hosts encountered and attacked (μ_h) and under different standard deviations of the within-host developmental conditions (SD_e). See Table 1 for the values of the simulation parameters used.

the proliferation mechanism of the polyembryonic mode of reproduction becomes less efficient in producing progeny. In such hosts, since monoembryonic females have a larger overall fecundity than their polyembryonic competitors, they can produce more progeny and always win the competition. The sensitivity analysis indicates a higher success rate of polyembryonic females when their overall egg complement approaches the egg complement of their monoembryonic counterparts (by increasing Ec_p up to $Ec_p=Ec_m$; Table 1). When Ec_p is below 5, monoembryony wins the competition whatever the size the hosts attacked. When $Ec_p > 8$, on the other hand, the proliferation mechanism of the polyembryonic females leads them to outcompete the monoembryonic individuals at all host sizes.

We also tested the sensitivity of the model outputs to the proliferation cost paid by the polyembryonic females. Eliminating the proliferation cost entirely shifted the host size threshold for establishment of polyembryony to the left. In other words, it increased the range of host sizes that allowed polyembryony to win the competition. As proliferation cost was increased, the success of polyembryony became restricted to larger and less variable hosts. At an even higher proliferation cost, polyembryony was outcompeted by monoembryony regardless of host size and variability (Fig. 2).

Further sensitivity tests showed that the constraints on polyembryony also increased as the monoembryonic competitors became more accurate in estimating the hosts' final size. When SD_m was reduced to zero (i.e. no host size-estimation errors were committed by monoembryonic wasps), polyembryony established only in the very largest hosts. With $SD_m \geq 18$, polyembryonic females won the competition under almost all conditions (Fig. 3).

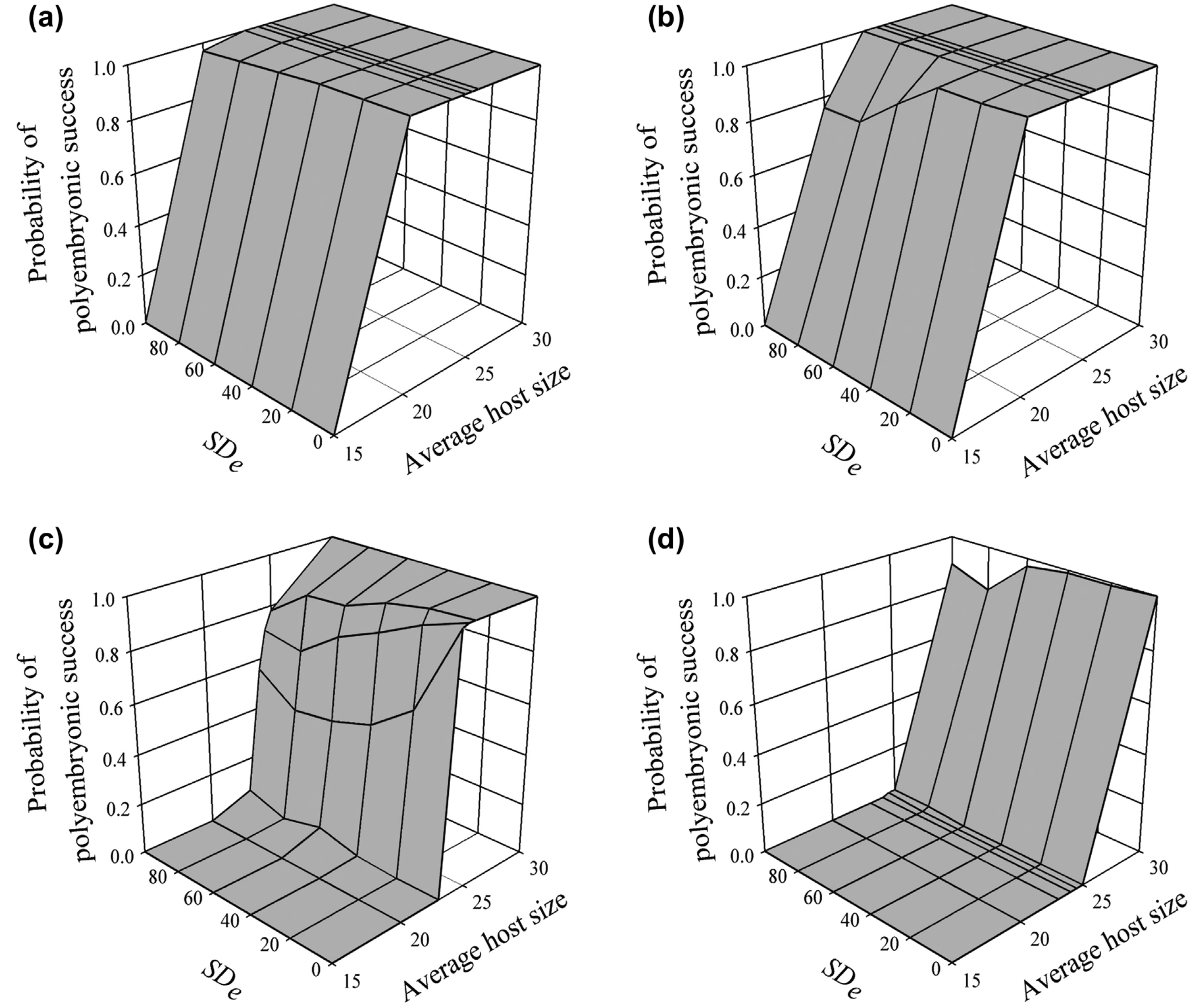

Figure 2. Results of the egg-limited model with different physiological cost associated with polyembryonic proliferation. Graphs are constructed as in Fig. 1, but the slope defining the proliferation cost (Table 1) was set to (a): 0.0, (b): 0.6, (c): 1.2 and (d): 1.4. Intermediate graphs are obtained with intermediate values (data not shown).

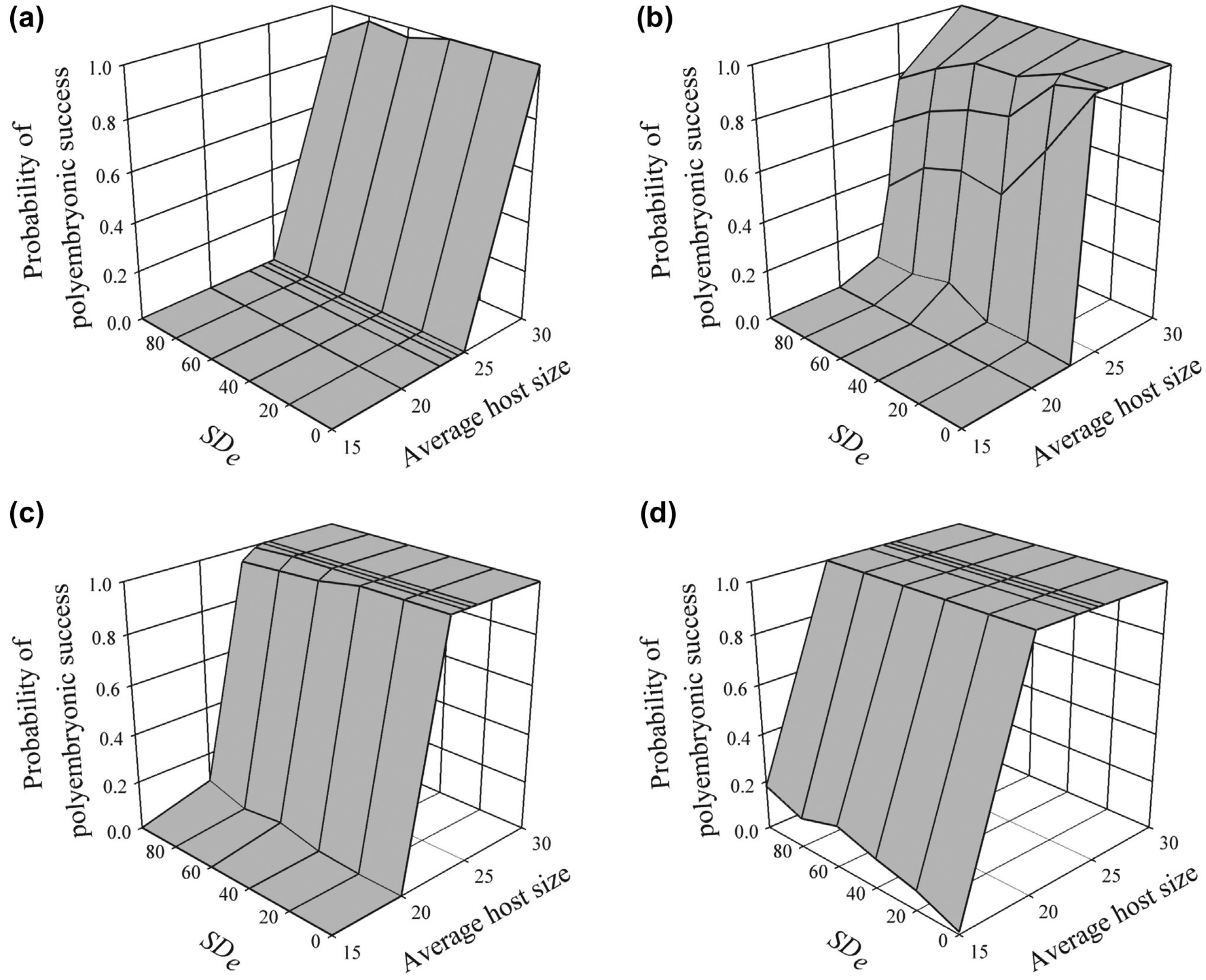

Figure 3. Results of the egg-limited model with different standard deviation of the error monoembryonic females make upon encountering a host to decide how many eggs to lay (i.e. SD_m; Table 1). Graphs are constructed as in Fig. 1, but with (a): $SD_m = 0$, (b): $SD_m = 6$, (c): $SD_m = 10$ and (d): $SD_m = 16$. Intermediate graphs are obtained with intermediate values (data not shown).

A strong hypothesis proposed to explain the low prevalence of polyembryony among parasitoid species is based on a potential loss of genetic variation as a result of the clonal mechanism involved in the proliferation process. Such a potential reduction in genetic variation among polyembryonic progeny would reduce individuals' survival under more variable, unpredictable environmental conditions. This hypothesis is supported by our model in most cases: when hosts are large and the variation in their quality/type is increased, polyembryonic females are indeed eliminated more often. However, such an effect seems somewhat smaller than the effect of host size (Fig. 1). Interestingly, host variability has the opposite (i.e. positive) effect on the success of polyembryony when average host size is slightly lower than the threshold that favors polyembryony ($\mu_h = 24$ in Fig. 1). A possible explanation is that introducing variability in host size under these parameter values randomly generates a small proportion of above-threshold hosts. Polyembryonic wasps reproduce efficiently in these hosts, and thus have a chance to eventually dominate the population and contribute offspring to the next generations. When mean host size is slightly above the threshold ($\mu_h > 24$), however, an opposite effect of host variability is observed.

The time-limited model

With the parameter values provided in Table 1, monoembryonic females always win the competition. The proliferation process of the polyembryonic wasps does not provide them with a competitive advantage over monoembryonic wasps, since monoembryonic females are not egg-limited anyway and can easily fill up all attacked hosts with eggs. This allows the monoembryonic females to always produce more progeny. In agreement with this interpretation, monoembryonic females in the time-limited model had a much higher realized lifetime fecundity than in the egg-limited model. For example, when ovipositing in large and uniform hosts ($\mu_h = 30$, $SD_e = 0.0$), monoembryonic wasps laid >300 eggs over ~9 foraging bouts on average during their lifetime. This greatly exceeds their egg complement in the egg-limited version of the model (100 eggs). As might be expected, increasing the risk of time limitation for polyembryonic females does not change the simulations' results: when polyembryonic females have lower survival probabilities and lower host encounter rates than their monoembryonic competitors, polyembryony is always excluded. In the model, not all the progeny that develop polyembryonically emerge from the host as adults, since females are paying a proliferation cost (Table 1). On the other hand, monoembryonic females can make mistakes in estimating the final size of the host they are attacking, eventually leading them to lay clutches of suboptimal sizes. Similarly to the egg-limited model, we relaxed these two types of cost to understand their effect on the model outputs. When the simulations are carried out with the same life-history traits for both types of females, but without the proliferation cost for the polyembryonic females, then polyembryonic females always win the competition. Reciprocally, if only the possible error of the monoembryonic females to estimate the final size of the hosts is eliminated, then monoembryony always wins. Finally, excluding both the proliferation cost for the polyembryonic females and the host size estimation mistake for the monoembryonic females leads to a draw: the frequency of winning the competition does not significantly differ from 0.5 for both types of females ($\chi^2 = 2.93$, df = 1, $p > 0.05$).

Literature survey

Our simulations suggest that polyembryony apparently faces fewer constraints under conditions of egg-limitation than under host/time-limitation. As an indirect test of this prediction, we compiled information on the lifetime egg production of polyembryonic parasitoids, and on their clutch sizes (the number of individuals that develop per host; see Supplementary material Appendix 1 Table A1). The ratio of lifetime fecundity over clutch size estimates how many hosts could potentially be parasitized by a female before it experiences egg limitation, had the species been monoembryonic (Table 3). For example, *Copidosoma floridanum* (Encyrtidae) females produce about 40 eggs throughout their life, yet clutch sizes in this species often exceed 1000 individuals (Ode and Strand 1995). Had this species been monoembryonic, it would have laid its whole egg complement in a single (necessarily very large) host, and would clearly be egg-limited in its next host encounters. As a counter-example, *Platygaster hiemalis* (Platygastridae) females produce more than 3000 eggs during their life, and lay clutches of two individuals only. Had the species been monoembryonic, it would have had sufficient eggs to parasitize >1500 hosts before becoming egg-limited. Based on Table 3 and on this reasoning, if the species were monoembryonic, the risks of egg-limitation would be highest within the Encyrtidae, intermediate within the Braconidae and quite low within the Platygastridae. Consequently, the life-history parameters of the polyembryonic Encyrtidae, and to a lesser degree also of the Braconidae, seem to be supported by our model's predictions.

A second prediction of our simulations is that small-bodied hosts constrain the evolution of polyembryony. To test this prediction we compared, whenever possible, the final body sizes of hosts parasitized by polyembryonic species with those parasitized by their monoembryonic congeners. We also compared the host/parasitoid body size ratio for these species to test for the possibility that low body size ratios could limit polyembryony. The family Encyrtidae does not include genera that represent both developmental modes, and the genus-level phylogeny is unknown. We therefore resorted to contrasting monoembryonic and polyembryonic encyrtid genera that attack hosts within the same insect order.

Supplementary material Appendix 1 Table A2–A4 list the developmental mode, body sizes and host sizes for the species covered by the literature survey, as well as the source references. The database compiled by Ulrich (2006) on parasitoid sizes and van Achterberg's (1993) monograph on Braconidae contained information on several species. We relied on additional publications on specific parasitoids to complement these data. Within the Encyrtidae (Supplementary material Appendix 1 Table A2), most polyembryonic species are koinobiont egg-larval parasitoids of Lepidoptera while monoembryonic species develop as idiobionts within Lepidoptera eggs (genus *Ooencyrtus*) or small pupae (genus *Parablastothrix*). Thus, monoembryonic species seem to exploit smaller-bodied hosts than their polyembryonic counterparts, which is in line with our prediction. Two encyrtid genera, the polyembryonic *Coelopencyrtus* and the monoembryonic *Cheiloneurus*, parasitize Hymenoptera. Here also, the polyembryonic genus utilizes larger hosts (bee larvae) than its monoembryonic relatives, which are hyperparasitoids of other small Encyrtidae and Dryanidae, hence supporting again our prediction. Two additional encyrtid genera, *Paratetracnemoidea* and *Encyrtus*, are monoembryonic parasitoids of coccids. These genera were not included in the comparison, since no polyembryonic parasitoids of coccids have been described.

Table 3. Ranges of lifetime fecundity and clutch sizes for polyembryonic parasitoids, and their expected risk of experiencing egg limitation if these species would have been monoembryonic.

Parasitoid family	Eggs produced by polyembryonic species	Clutch size	Risk of egg limitation
Encyrtidae	40–200	40–1200	highest
Braconidae	50–250	17–280	intermediate
Platygastridae	650–3300	2–15	lowest

All *Macrocentrus* (Braconidae) species are koinobionts that parasitize larvae of Lepidoptera and often attack more than one host species. The size that the holomentabolous larvae achieve just before metamorphosis determines their final size as adults (Callier and Nijhout 2013). We therefore used the mean adult size of the most common host of each *Macrocentrus* species as a proxy of its final host size (Supplementary material Appendix 1 Table A3). Contrary to the predicted pattern, polyembryonic species of this genus develop on hosts of smaller adult size (mean ± SE wingspan of adult hosts: 19.00 ± 4.37 mm) than their monoembryonic gregarious congeners (wingspan of adult hosts: 31.44 ± 4.53 mm). The genus *Platygaster* (Platygastridae) uses gall midges as hosts. Hosts of polyembryonic *Platygaster* species are very similar in size to the hosts of the monoembryonic gregarious species within the genus (body lengths: 3.48 ± 0.57 and 3.43 ± 0.30 mm for adult hosts of polyembryonic and monoembryonic species, respectively; Supplementary material Appendix 1 Table A4). We conclude that the predicted correlation between small host size and monoembryony is partially supported by the data available on Encyrtidae, but not by the data on Braconidae and Platygastridae. This conclusion remains unchanged when the ratios of final host size to parasitoid size are compared between polyembryonic and monoembryonic species.

Discussion

Polyembryony is a highly efficient way to generate many offspring from a small number of eggs, so why is it quite rare among parasitoids? Using Monte Carlo simulation models, we reduced selective benefits and increased costs associated with polyembryony, in order to explore constraints on its evolution.

The benefit of avoiding egg limitation

We found that a high risk of egg limitation favored polyembryony, and that this mode of development was outcompeted by monoembryony when the risk of egg limitation was reduced. This was reflected by the fact that polyembryony won the competition in egg-limited populations under several simulated conditions, but invaded time-limited populations in one scenario only: when the cost of proliferation was removed (Table 2; tests 1 and 5). In egg-limited populations, the success of polyembryony was lower in smaller hosts than in larger ones (Table 2, test 2). This is because smaller host size reduces the risk of egg depletion for monoembryonic females and the proliferation process is less effective in smaller hosts than in larger ones. These findings seem to support the hypothesis that polyembryony can alleviate egg limitation in parasitoids, but is not expected to evolve when eggs are unlimited in a time-limited scenario. Empirical studies actually suggest that time and hosts are usually more limiting than eggs for parasitoids (West and Rivero 2000, Rosenheim et al. 2008). According to the predictions of our models, this seems to be consistent with the rarity of polyembryony within parasitoid Hymenoptera.

It is noteworthy that host size affected the simulations' outcome only within a narrow range of conditions (egg-limitation and $4 < EC_p < 8$), suggesting a restricted role to host size as a selective agent. This may be because polyembryonic parasitoids generate more offspring in larger hosts, but also suffer a higher proliferation cost. Additionally, simulated monoembryonic wasps also pay a fitness cost when developing in small hosts. SD_m (the standard deviation of the host-size estimation error by monoembryonic females) was considered a constant independent of host size in the simulations. Consequently, females are predicted to oviposit more superfluous eggs in small hosts than in large ones, leading to higher mortality of monoembryonic offspring in small hosts. In support of this interpretation, we found that monoembryonic females dominated a wider range of host sizes when SD_m was reduced (Fig. 3).

We performed a literature survey to assess whether lineages of polyembryonic parasitoids indeed face a high risk of egg limitation. This seems to be the case for polyembryonic species of the Encyrtidae family, which produce moderate numbers of eggs but have large offspring broods, and parasitize larger-bodied hosts than their monoembryonic relatives. This suite of life-history traits may have led to egg limitation in some ancestral encyrtids, and could have facilitated the evolution of polyembryony in the lineage by allowing small parasitoids to attack large hosts.

We found less evidence for potential egg limitation in polyembryonic species of *Macrocentrus* (Braconidae), which have fairly small clutch sizes and parasitize smaller hosts than their gregarious monoembryonic congeners. A caveat regarding our comparative dataset of host sizes is that we used the wingspan of the adult lepidopteran hosts of *Macrocentrus* as a proxy of final host size, whereas the parasitoid develops in larval stages. The estimation of host size is further complicated by the facts that many *Macrocentrus* species attack several lepidopteran host species (van Achterberg 1993), at different larval stages (Haeussler 1932), thus with different potential sizes. Moreover, parasitized hosts are often manipulated by the parasitoids to feed more and grow larger than healthy hosts, or even to molt into an additional larval instar (Li et al. 1999). Hence, detailed information on final host sizes is needed to better test the model's prediction.

The high fecundity and low clutch sizes within the genus *Platygaster* (Platygastridae) do not suggest a high risk of egg limitation, which appears to be in contradiction with the simulations' predictions. Polyembryony in this genus involves relatively small clones (Table 3), and one species is even capable of both monoembryonic and polyembryonic development (Leiby and Hill 1923). These data suggest that polyembryony in the Platygastridae has diverged to a lesser extent from monoembryony, compared to other parasitoid families. Reduction of within-brood competition was suggested to be a major driver of the initial evolution of polyembryony (Segoli et al. 2010b). However, this was not included in our model, as we focused on gregarious wasps with no aggression within broods. This may be the main reason why the life-history of *Platygaster* does not confirm the simulation's predictions.

The benefit of optimizing clutch size

We also considered the hypothesis that monoembryony will outcompete polyembryony when monoembryonic parasitoids can accurately adjust their clutch sizes to the carrying capacity of their hosts. We thus predicted that eliminating errors of host size mis-estimation in the simulation would favor monoembryony and limit the spread of polyembryony. This prediction was tested and confirmed for both versions of the model (Table 2; test 3). It should be noted that experimental studies, in two polyembryonic *Copidosoma* species, found that proliferation occurs at early developmental stages (Segoli et al. 2009), at which the final size of the host cannot yet be foreseen by the developing progeny. The clutch is later adjusted to the host's carrying capacity through larval mortality, modification of larval body size or even manipulation of host size (Saeki et al. 2009, Segoli et al. 2010a, Saeki and Crowley 2013). These studies suggest that the major benefit of the polyembryonic proliferation process, at least in some species, is in alleviating egg limitation rather than in optimizing the clutch size.

The cost of low genetic variation

Increased environmental heterogeneity (variation in the type/quality hosts, Table 3; test 4) favored gregarious monoembryony at the expense of polyembryony, as predicted by Craig et al. (1995, 1997). However, this effect was much weaker than that of host size, at least for the range of parameter values tested in our simulations. When host size was slightly below the threshold that favored polyembryony, we even found the opposite trend, namely polyembryony was somewhat more successful on variable hosts than on hosts of uniform phenotype (Fig. 1). The most likely reason for the weak effects of host variability is that polyembryonic development indeed reduces the genetic variability within hosts, but the population-level genetic variation is preserved, as suggested by Hardy (1995). The cost of a potential reduction in within-host genetic variability might thus be too slight to explain the rarity of polyembryony as an alternative to gregarious monoembryony. Furthermore, solitary monoembryonic parasitoids are expected to suffer similar low genetic variability costs compared to polyembryonic species, because oviposition in an unsuitable host results in zero progeny in both cases. Thus, the low genetic variability cost is likely an even weaker constraint on polyembryony when competing with monoembryonic solitary development.

The proliferation cost

Finally, removal of the proliferation cost paid by the polyembryonic females allowed them to invade the simulated populations, even under the restrictive condition of time limitation (Table 2; test 5). Under the egg-limited scenario, reducing the proliferation cost allowed polyembryony to establish in a wider range of host sizes, and in more variable hosts (Fig. 2). Such a cost of proliferation thus appears to be an important barrier to polyembryony. By increasing host size in the simulation, we both increased the egg limitation constraint (Table 2; test 2), and increased the proliferation cost (Table 2; test 6). Higher egg limitation constraints favor polyembryony, while a higher proliferation cost acts against it. Thus, the two mechanisms predict opposite effects of final host size on the success of polyembryonic development. The simulated polyembryonic individuals consistently improved in performance when host size was increased, because the bigger clutches that can develop in large hosts compensated for the increase in proliferation cost. This suggests that the risk of egg limitation plays a larger selective role than the effect of proliferation cost.

The simulations also generate some insights regarding the relative importance of the proliferation cost for the polyembryonic females, versus the host-size estimation mistakes potentially made by their monoembryonic counterparts. When both of these costs were excluded from the time-limited model, both monoembryonic and polyembryonic females were equally successful, provided that they had the same survival probability and host finding efficacy. Introducing a host-size estimation mistake only for the monoembryonic females allowed the polyembryonic wasps to always win (Table 2; test 3), whereas adding the proliferation cost as well for the polyembryonic females enabled the monoembryonic wasps to take over (Table 2; test 5). This implies that the proliferation cost for the polyembryonic females did not simply balance the host-size estimation mistake for their monoembryonic competitors, which would have made both strategies equally successful. Rather, it had a stronger effect than the host size mis-estimation cost for the monoembryonic females (at least for the parameter values explored in the simulations). Thus, when the proliferation cost was added to a model that included host size mis-estimation costs, the competition outcome switched from complete polyembryony to complete monoembryony.

Conclusion

The simulations in this study allowed us to quantify and compare the effects of several life-history variables as limiting factors to the evolution of polyembryony. While a wide range of situations favored the evolution of monoembryony over polyembryony, the most salient factors were 1) a higher risk of host/time limitation than of egg limitation, 2) a high physiological cost of polyembryonic proliferation, and 3) a high accuracy of monoembryonic females in estimating the final host size. Two other potential limiting factors to polyembryony, host size and host variability, appear less important (at least for the parameter ranges explored here). Our simulations assumed equal initial frequencies of monoembryonic and polyembryonic females, i.e. did not consider possible frequency-dependent selection against the initial spread of rare polyembryonic mutants. Such selection might constrain the evolution of polyembryonic even further. The models' predictions can be qualitatively tested by comparing life-history traits of polyembryonic species and their monoembryonic relatives. Unfortunately, the small number of species for which this information is currently available precludes statistically rigorous testing. Additional laboratory and field studies on the phylogeny, ecology and life-history of polyembryonic parasitoids are needed to establish the relative importance of these constraints. Simulated competitions between polyembryonic and monoembryonic solitary wasps are an additional promising future extension of the present work. Such simulations would differ from the current modeling framework in several aspects. First, they should include a different genetic variation cost for polyembryonic wasps. This is because both types of parasitoids lose their entire brood if laid in an unsuitable host, but the number of offspring lost by polyembryonic parasitoids is much larger than by solitary ones. Second, the simulations need to incorporate the cost of within-brood aggression in the monoembryonic case, while polyembryonic wasps do not pay this cost. Finally, solitary parasitoids do not make host size estimation errors and are unlikely to become egg-limited as they lay a single egg per host.

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Supplementary material (available online as Appendix oik-05479 at <www.oikosjournal.org/appendix/oik-05479>). Appendix 1.