REVIEW

The evolution of polyembryony in parasitoid wasps

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Abstract

Polyembryony has evolved independently in four families of parasitoid wasps. We review three main hypotheses for the selective forces favouring this developmental mode in parasitoids: polyembryony (i) reduces the costs of egg limitation; (ii) reduces the genetic conflict among offspring; and (iii) allows offspring to adjust their numbers to the quality of the host. Using comparative data and verbal and mathematical arguments, we evaluate the relative importance of the different selective forces through different evolutionary stages and in the different groups of polyembryonic wasps. We conclude that reducing the cost of egg limitation is especially important when large broods are favoured. Reducing genetic conflict may be most important when broods are small, thus might have been important during, or immediately following, the initial transition from monoembryony to polyembryony. Empirical data provide little support for the brood-size adjustment hypothesis, although it is likely to interact with other selective forces favouring polyembryony.

Introduction

Polyembryony is a unique mode of development that involves the production of several genetically identical embryos from a single egg through clonal division. Occasional polyembryony that results in twinning occurs in almost all animal taxa. By contrast, obligatory polyembryony (which occurs in every reproductive event) is relatively rare and occurs in fewer taxonomic groups (e.g. flatworms, bryozoans, insects, mammals; reviewed in Patterson, 1927; Craig *et al.*, 1997). It has been suggested that polyembryony is costly, because it clones an unproven genotype (different from that of the parent) at the expense of genetic diversity within a brood (Craig *et al.*, 1995, 1997). In spite of this likely disadvantage, polyembryony has evolved in at least four families of parasitoid wasps (Hymenoptera). One of these polyembryonic lineages has become highly successful, diversifying to include hundreds of species (Guerrieri & Noyes, 2005). Polyembryony has probably evolved independently in each of these families, as most species within

each family are monoembryonic, and the families are not closely related to each other within the Hymenoptera (Strand, 2003; Grbic, 2003). This raises the questions: what are the main selective forces favouring polyembryony in parasitoid wasps, and why has it evolved in some groups but not in others?

In this paper, we attempt to provide a synthetic, concise review of several hypotheses for the evolution of polyembryony. We start by presenting general information on polyembryonic parasitoids and discuss possible preadaptations for this developmental mode. We then introduce three nonmutually exclusive hypotheses for possible selective forces favouring polyembryony in this group (Table 1). We evaluate relevant empirical data and consider some simple models that explore the plausibility and relative importance of the different hypotheses through different evolutionary stages and in the different groups of polyembryonic wasps.

Polyembryony in parasitoid wasps

Polyembryony is known from the hymenopteran families Platygasteridae, Braconidae, Dryinidae and Encyrtidae (Craig *et al.*, 1997; Strand, 2003). In the process

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Table 1 Main hypotheses for the evolution of polyembryony in parasitoid wasps.

Hypothesis	Assumptions	Predictions	Test of predictions	Relative importance
Reduce the cost of egg limitation	Reproductive success of female parasitoids is limited by the number of eggs that they can produce	Polyembryonic wasps can potentially produce more offspring than monoembryonic wasps. Polyembryony is accompanied by increased brood size per host, increased host size/quality and reduced fecundity Gregarious ancestor	Comparative analysis demonstrates that polyembryonic encyrtids can potentially produce more offspring, have larger broods and lower fecundities than monoembryonic species*	Important during the initial transition from monoembryony to polyembryony, and in maintaining and accelerating the degree of polyembryony in species with large broods: e.g. genus *Copidosoma*
Reduce genetic conflict between offspring	Parent–offspring conflict depends on relatedness among offspring	High cooperation between offspring of polyembryonic compared to monoembryonic species Cooperation according to relatedness Solitary ancestor	Evolution of a soldier caste in encyrtid species. Soldiers attack according to relatedness†	Important during, or immediately following, the initial transition from monoembryony to polyembryony, and in species with small broods, e.g. genus *Platygaster*
Adjust brood size to host carrying capacity	Large variation in hosts' final carrying capacity cannot be foreseen by the mother but may be perceived by the offspring during their development	Offspring adjust their numbers according to host size/quality	Weak evidence for adjustment of primary clone size‡	Likely to interact with the other selective forces when secondary rather than primary brood sizes are adjusted

*Current paper, Box 1.
†Giron *et al.* (2004) for *Copidosoma floridanum*, Segoli *et al.* (2009b) for *Copidosoma koehleri*.
‡Saeki *et al.* (2009) for *Copidosoma bakeri*, Segoli *et al.* (2010) for *C. koehleri*.

of polyembryonic development, a primary cell mass (morula) divides into a mass of developing embryos (polymorula). Embryos develop into larvae that actively feed on the host tissues before they pupate (Grbic *et al.*, 1998). The number of embryos developing from a single egg through polyembryony varies substantially among species. In platygasterids that parasitize eggs of gall-midges (Diptera), clone size is small, with 2 to approximately 10 individuals developing from each egg (Leiby & Hill, 1923, 1924; Leiby, 1929). In the species *Platygaster hiemalis*, some eggs are monoembryonic, whereas others develop into two embryos. This was suggested to represent the most primitive form of polyembryony (Leiby & Hill, 1923). In polyembryonic braconids that parasitize moth larvae (Lepidoptera), clone size ranges from 2 to 50, depending on species (Daniel, 1932; Parker, 1931; Orr *et al.*, 1994; White & Andow, 2008). In polyembryonic dryinids that parasitize nymphs of leafhoppers (Hemiptera), clone size reaches 60 (Kornhauser, 1919). In polyembryonic encyrtids that parasitize eggs of Lepidoptera and some Hymenoptera, clone sizes vary from 2 (e.g. *Ageniaspis citricola*, Zappalà & Hoy, 2004) to several dozens (e.g. *Ageniaspis fuscicollis*, Kuhlmann *et al.*, 1998; *Copidosoma koehleri*, Segoli *et al.*, 2009a), and up to hundreds or even thousands of individuals (e.g. *Copidosoma floridanum*, Strand, 1989; Ode & Strand, 1995; *Copidosoma bakeri*, Saeki *et al.*, 2009). In some encyrtid parasitoids, a subset of the embryos from a single egg develops into specialized soldier larvae with slim bodies and large mandibles. Soldiers attack intra- and inter-specific competitors inside the host and never pupate or emerge from the host as adults (Cruz, 1981, 1986; Giron *et al.*, 2004; Segoli *et al.*, 2009b). The large clone sizes and the development of a soldier caste suggest that in this family, polyembryony has reached its most specialized form.

Common life history features of polyembryonic wasps

Parasitoid life histories can be divided into several categories. One distinction is between endoparasitoids that develop within the body of their hosts, feeding from the inside, and ectoparasitoids that feed on the host externally. Another important distinction is between koinobiont parasitoids that allow their hosts to continue developing after parasitism (for example egg-larval parasitoids that parasitize the host egg, with their offspring emerging out of the host larva) and idiobionts that arrest host development. Finally, in gregarious parasitoids several larvae can complete their development on the body of a single host, whereas in solitary parasitoids only one larva survives (Godfray, 1994).

Interestingly, all polyembryonic wasps fall into the same category: they are relatively small in size (adult body size usually ≤ 1 mm), endoparasitoids and koinobionts (mostly egg-larval parasitoids). Because

Box 1: Potential number of offspring for polyembryonic vs. monoembryonic wasps

Polyembryony may reduce the cost of egg limitation by allowing small females to produce many offspring. For example, the wasp *C. floridanum* that parasitizes moth eggs is about 1 mm long. A female may parasitize multiple hosts, each giving rise to about 1200 offspring and up to 3000. It is reasonable to assume that a wasp of this size would not have been able to produce that many offspring if it were not polyembryonic. To further demonstrate this point, we gathered life history data for 23 monoembryonic and seven polyembryonic encyrtids (Fig. 1). The potential number of offspring for polyembryonic species (full triangles) was estimated as their mean fecundity (empty triangles) multiplied by mean clone size. The potential number of offspring for females of monoembryonic species (empty circles) was simply estimated as their mean lifetime fecundity. The data were not corrected for phylogeny, as a full phylogenetic hypothesis for this family is not available (J. Noyes, pers. comm.). The mean number of potential offspring per female in polyembryonic species (30 334 ± 18 408 [SE], $n = 7$) is substantially larger (by up to several orders of magnitude) than in monoembryonic species (175.5 ± 25.5, $n = 23$; Mann–Whitney U test, $U = 5$, $P < 0.001$). This difference is mediated through an increase in brood size per host, as predicted by the egg limitation hypothesis (see text and Box 2) (mean brood size = 506.3 ± 296.7 offspring, $n = 7$, for poly- and 8.6 ± 2.5 eggs, $n = 9$ for mono-embryonic gregarious species, Mann–Whitney U test, $U = 10.5$, $P = 0.023$). In further accord with predictions, the mean fecundity of polyembryonic species (83.6 ± 21.3, $n = 7$) is lower than that of monoembryonic species (175.5 ± 25.5, $n = 23$; Mann–Whitney U test, $U = 36$, $P = 0.029$). This reduction in fecundity might have preceded polyembryony, increasing the selective pressure to overcome egg limitation. Alternatively, it might have evolved secondar-ily, suggesting that once polyembryony is established, it may allow females to reduce their costs of egg production. However, this result should be treated with caution, as the difference is not large, and data were not corrected for phylogeny. Data for monoembryonic species were taken from: Avidov *et al.*, 1967; Berlinger, 1973; Odebiyi & Bokonon-Ganta, 1986; Blackburn, 1991; Hanks *et al.*, 1995; Rosenheim & Hongkham, 1996; Lane *et al.*, 1999; Sagarra *et al.*, 2001; Geden *et al.*, 2003; Ceballo & Walter, 2004; Chong & Oetting, 2006; Gulec *et al.*, 2007; Sandanayaka *et al.*, 2009 and an unpublished database compiled by Werner Ulrich (pers. comm.). Data for polyembryonic species were taken from: Kfir, 1981; Strand, 1989; Wang & Laing, 1989a,b; Ode & Strand, 1995; Kuhlmann *et al.*, 1998; Harvey *et al.*, 2000; Zappalà & Hoy, 2004; Saeki *et al.*, 2009; Segoli *et al.*, 2009a. Additional unpublished data were obtained from P. Crowley, C. Fox, P. Ode and L. Zappalà.
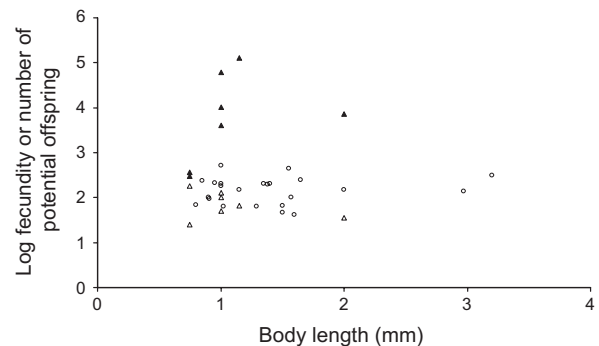

Fig. 1 Fecundity and potential number of offspring for polyembryonic vs. monoembryonic encyrtid wasps. See text for key.

embryonic division takes place inside the host, polyembryonic species are generally gregarious (although their ancestor might have been solitary). These common characteristics may be viewed as preadaptations to the evolution of polyembryony: endoparasitoids develop within the nutrient-rich body of another insect, where protection from desiccation and provisioning of a nutrient-rich yolk are not required. Unconstrained by a rigid chorion, the embryos have the potential to increase in volume significantly during embryogenesis, as occurs during polyembryonic development. Koinobiont development allows for a long developmental period, which may also be needed to complete polyembryonic division. Thus, these traits may be important in providing a setting within which polyembryony had an opportunity to evolve (Ivanova-Kasas, 1972; Strand, 2003; Grbic, 2003).

From a slightly different perspective, these shared characteristics may also shed light on the main constraints acting on polyembryonic wasps: in gregarious parasitoids, there is a strong trade-off between the number and size of offspring per host. Small body size may limit the number of eggs that a female can produce.

In spite of the potential advantages of attacking a defenceless young host, it may decrease the ability of the ovipositing female to adjust clutch size to the future carrying capacity of the host. A lengthy development within the host may also reduce the probability that the host, and thus the parasitoids developing within the host, survive throughout development. Finally, a lengthy development may increase the number of potential competitors, as the time window for attack by additional parasitoids is relatively long. In the next sections, we discuss how polyembryony may potentially enable a parasitoid to overcome these difficulties.

Hypothesis I: reducing the cost of egg limitation

Reducing the cost of egg limitation is perhaps the most immediate advantage of polyembryony. A female that is limited by the number of eggs that she can produce during her lifetime, or at a specific stage of her life, may overcome this limitation by laying eggs each of which produces several offspring. Nevertheless, this explanation

Box 2: Under which conditions is the cost of egg limitation expected to be high?

Egg limitation may be a key factor that affects reproductive output of parasitoid wasps. This raises the question: why have not all endoparasitoids evolved polyembryony to overcome egg limitation? A possible answer is that only under certain circumstances is egg limitation prevalent enough to offset the costs associated with the reduction in offspring variability and therefore, to select for this novel developmental mode. For example, under some circumstances optimal clutch size (i.e. the clutch size that maximizes the fitness of a whole clutch, Lack, 1947) may exceed the number of eggs a female can produce. In such a case, even a female that succeeded in finding only a single host during her entire period of reproduction would still be egg limited, because she would be unable to produce the optimal-sized clutch for that host. We demonstrate this argument using a simple optimization model.

The model first calculates the optimal clutch size for a monoembryonic gregarious parasitoid female while initially assuming that each female has an unlimited number of eggs (no egg limitation). For simplicity, we assume that each female can only locate one host during its lifetime. This makes our model conservative, because females will be even more prone to egg limitation if they are able to locate many hosts. We assume that clutch size has a negative effect on the fitness of each offspring. This effect may be mediated through a decline in the offspring's ability to locate and successfully parasitize hosts. Following Skinner (1985), we model such a decline in individual fitness, w, as follows:

$$w = (1 - c/h)^a \qquad (1)$$

where c is clutch size; h is host quality (as it increases the negative effect of clutch size on the fitness of an individual offspring is reduced); and a is the shape parameter of the fitness function (as it increases, the negative effect of clutch size on fitness increases). The fitness of the whole clutch is calculated by multiplying clutch size by individual fitness:

$$w_c = c(1 - c/h)^a \qquad (2)$$

Setting $dw_c/dc = 0$ and solving for c yields an optimal clutch size (that maximizes clutch fitness):

$$c^* = h/(1 + a) \qquad (3)$$

Thus, optimal clutch size is positively affected by host quality while the amount of host resources allocated to a single offspring is constant:

$$h/c^* = (1 + a) \qquad (4)$$

Constant allocation to each offspring is a common prediction of size-number trade-off models (Mayhew & Glaizot, 2001).

We next calculate the expected fecundity, f, of females emerging from optimal clutches:

$$f = b(1 + a) \qquad (5)$$

where $(1 + a)$ represents resource allocation per offspring (see eqn 4) and b is a constant describing the increase in fecundity per unit of host resources. In this way, we separate the effect of clutch size on offspring fecundity (eqn 5) from the effect of clutch size on other components of offspring fitness (eqn 1). Recall that we are looking for the conditions under which optimal clutch size exceeds fecundity. This condition will be met when c^* is larger than f, i.e.:

$$h > b(1 + a)^2 \qquad (6)$$

Figure 2 shows the effect of clutch size on individual fitness (top) or the fitness of the whole clutch (middle), and the effect of host quality on optimal clutch size and fecundity (bottom) for fecundity constant $b = 10$, and for different values for the shape parameter (a) of the fitness function. The model predicts that optimal clutch size increases with host quality, whereas fecundity per female offspring remains constant. Optimal clutch size exceeds fecundity when host quality surpasses a certain threshold. Above this threshold, females that produce optimal clutches will have daughters that cannot produce optimal clutches, because of the size-number trade-off. The model predicts that this form of egg limitation is more likely, and thus polyembryony is more beneficial, as: (i) host quality (h) increases; (ii) fecundity per resource unit (b) decreases; and (iii) the shape parameter (a) decreases, resulting in larger optimal clutches.

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has received little attention (see Godfray, 1994; Craig *et al.*, 1997). One possible reason for the limited attention given to this hypothesis is the debate regarding the importance of egg limitation relative to time/host limitation, where egg limitation was often claimed to be negligible (e.g. Sevenster *et al.*, 1998; Ellers *et al.*, 2000). However, both theoretical developments (Rosenheim 1996; Mangel & Heimpel, 1998; Rosenheim *et al.*, 2010) and empirical evidence suggest that egg production may constitute a major limiting factor and thus should not be ignored. Empirical evidence includes dissections of field-collected females (Rosenheim *et al.*, 2008), estimates based on host encounter rate vs. the rate of egg maturation, behavioural responses to the risk of egg limitation (reviewed in Heimpel & Rosenheim, 1998) and data on sex ratios (West & Rivero, 2000). Polyembryony may allow female parasitoids to overcome egg limitation, thereby allowing these wasps to occupy a novel life history niche: a small body size with many offspring. Indeed, despite of their small size, the number of potential offspring for polyembryonic wasps is substantially larger than for monoembryonic wasps (Box 1).

The risk of egg limitation may be especially important under conditions that select for large parasitoid broods (Box 2). For example, hosts of large size or high quality may accommodate many parasitoids, thereby favouring large broods (Skinner, 1985; Mayhew & Glaizot, 2001).

Polyembryony may allow small wasps that would otherwise be limited by their low fecundities to exploit large hosts in an optimal way (Godfray, 1994). An alternative means of exploiting a relatively large host by parasitoids with limited fecundities is found in wasps of the genus *Melittobia* parasitizing prepupae of solitary bees and wasps, where each host is consumed successively by two generations of offspring (Matthews *et al.*, 2009). Another important factor determining optimal brood size is the relationship through which individual fitness is reduced with increasing offspring number (Skinner, 1985; Stearns, 1992; Box 2). When the survival of offspring, or their ability to locate and parasitize hosts, is critically dependent on parasitoid body size, the brood size that maximizes long-term reproductive returns is predicted to be relatively small. In contrast, when these factors do not strongly depend on parasitoid body size, large broods may be favoured. For example, body size may be less important in egg parasitoids that do not need to fight and overcome their hosts, compared to parasitoids of larval or adult stages. While the conventional view has been that body size is a strong determinant of parasitoid fitness in nature, it may be that in some cases a very large number of small, searching offspring may be a more effective strategy of locating low density hosts than having one or a few much larger offspring. Under this view, polyembryony might be favoured evolutionarily for the same reason that the production of a very large number of tiny male gametes is thought to lead to a higher probability that

female gametes are discovered and fertilized (Iyer & Roughgarden, 2008). Additionally, lengthy development within the host in koinobiont parasitoids may reduce host (and thus parasitoid) survival, independent of parasitoid body size. Price (1972) suggested that monoembryonic wasps may offset low host survival probabilities by evolving high fecundities and parasitism rates. Polyembryony, by releasing females from egg limitation, may further allow compensation for low host survival through the production of many offspring per host.

Based on the aforesaid, we infer that polyembryony as a means of overcoming egg limitation could have been important both at the initial transition from monoembryony to polyembryony, and in maintaining and increasing the number of embryonic divisions after polyembryony had been established. The importance of polyembryony as a means of reducing the cost of egg limitation is likely to be amplified by increased host size/quality, increased brood size and reduced parasitoid body size. In particular, this selective force is likely to be important in species with extremely large broods, such as members of the encyrtid genus *Copidosoma*. For example, the life history of the wasp *C. floridanum* seems to be in accordance with the aforementioned predictions: it has a small body (approximately 1 mm) and parasitizes relatively large hosts (plusiine moths, Lepidoptera). The wasps develop as the host passes through five instars, and each host gives rise to about 1200 offspring (Ode & Strand, 1995). Egg limitation may play a smaller role in
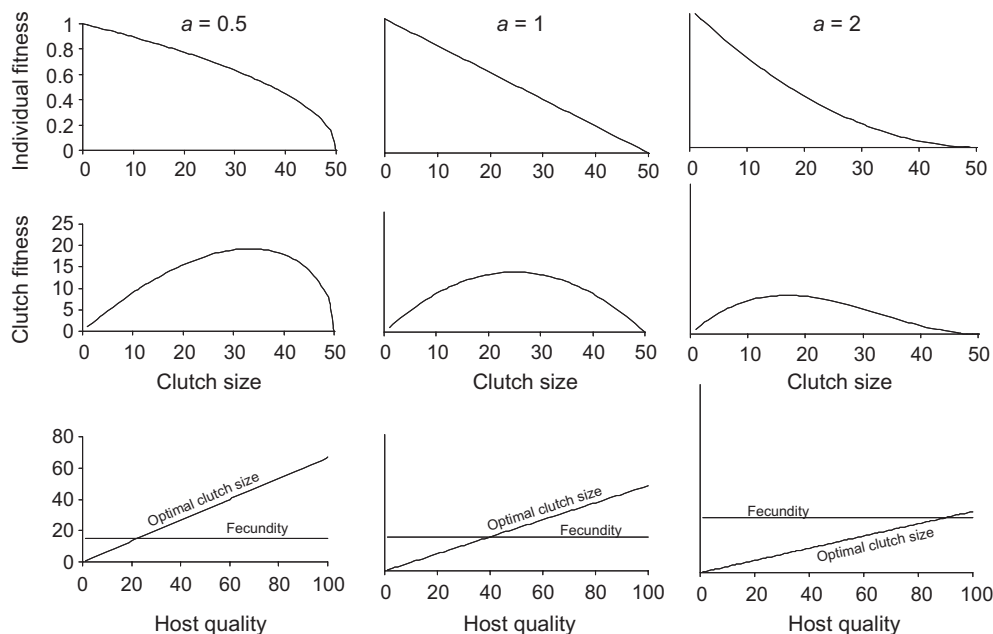

Fig. 2 The effect of clutch size on individual fitness (top) or the fitness of the whole clutch (middle), and the effect of host quality on optimal clutch size and fecundity (bottom) for fecundity constant $b = 10$, and for different values for the shape parameter (a) of the fitness function.

polyembryonic platygasterids or some braconids with small broods. For example, polyembryonic species of the genus *Platygaster* produce clones of 2–10 individuals, perhaps because they are restricted by the small size of their dipteran hosts.

Hypothesis II: reducing genetic conflict among offspring

The second hypothesis is based on the common assumption that optimal clutch size for parents is generally larger than it is for their offspring. This is because parents are selected to maximize the fitness of the whole clutch, whereas each offspring is selected to maximize its own fitness, even at the expense of its siblings (Trivers, 1974; Godfray & Parker, 1991). As the relatedness among offspring increases, cooperation is expected to increase and aggression to decrease (Hamilton, 1963). Polyembryony may be favoured because it eliminates parent–offspring conflict, allowing parents to produce optimally sized clutches while promoting cooperation among siblings (Godfray, 1994). Nevertheless, it maintains genetic variation among broods, and this provides an advantage over strictly asexual modes of reproduction such as parthenogenesis (Kirkendall & Normark, 2003).

The advantage of increased cooperation was shown to be negated in some cases by increased competition among the interacting siblings (reviewed in West *et al.*, 2002). For example, the advantage of reduced aggression among male fig-wasps is offset by intense local competition for access to females within the fig (West *et al.*, 2001). However, this may not be the general case for parasitoid larvae, as cooperation among siblings may allow them to exploit a larger proportion of the host resources or to exploit the host more efficiently (Godfray,
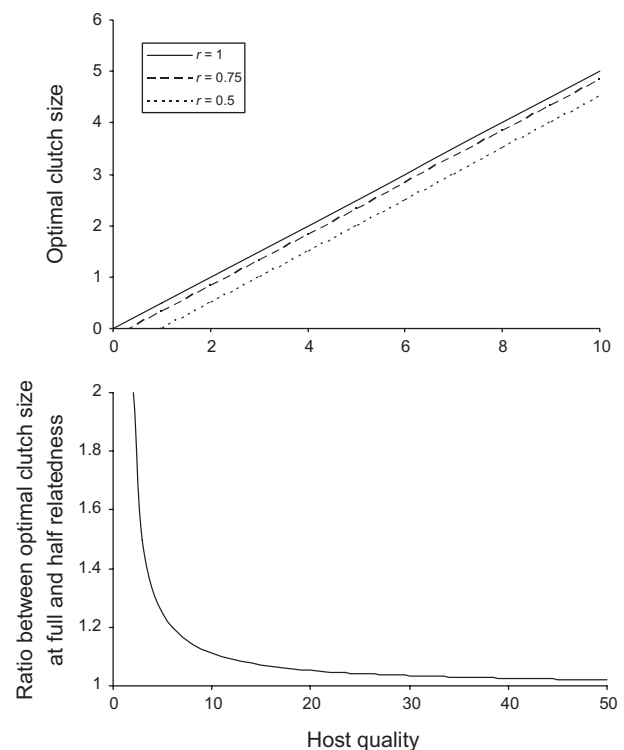

Fig. 3 Optimal clutch size from the offspring's point of view as a function of host size for relatedness values of 1, 0.75 and 0.5 (top), and the ratio between optimal clutch size at full relatedness and at half relatedness (bottom).

1994; Lalonde, 2005; Hoffman, 2009). For example, the production of a soldier caste in polyembryonic wasps provides evidence for the advantages of strong

Box 4: Can polyembryony facilitate the transition from (siblicidal) solitary to (tolerant) gregarious developmental mode?

To answer this question we developed a simple genetic model based on Godfray (1987). The critical condition for the successful invasion of a rare, mutant allele coding for nonsiblicidal behaviour is framed in terms of the threshold fitness value of a parasitoid developing in a clutch of two eggs, $f(2)$. If we define $f(1) = 1$, the critical condition under monoembryonic development with random sex allocation is $f(2) > 1$ (Godfray, 1987); that is, the fitness of a parasitoid developing in a clutch of two eggs must exceed the fitness of a parasitoid developing alone. This is a strikingly stringent condition, leading to the suggestion that transitions to gregarious development may be quite difficult to achieve. The stringency of the condition is heightened further if the allele for nonsibilicidal behaviour is expressed as a recessive trait. Sex allocation behaviours that increase the average relatedness between offspring, such as female biased sex ratios or the production of single sex broods, have been shown to relax the stringency of the condition somewhat, with the $f(2)$ threshold varying from 0.78 to 1 depending on the sex ratio and on the proportion of mixed sex broods (Ode & Rosenheim, 1998). In our model, we assumed that each female lays a single egg (either a haploid male egg or a diploid female egg) within a host, and that each egg divides polyembryonically to produce a brood of two identical twins. If the original egg carries a dominant allele for tolerance, both offspring will carry this allele and no fighting will occur. Otherwise, fighting will occur and only one of two offspring will survive. In both cases, larval behaviour (tolerance vs. fighting) is expressed without regard to the relatedness of other larvae found within the same host; thus, no kin recognition is required. Note that under polyembryony and in the absence of superparasitism (which can act against the spread of nonsiblicidal alleles), a tolerant mutant always shares a host with an additional tolerant individual and thus will never be eliminated by an individual carrying a siblicidal allele. In this case, the threshold for the spread of a nonsiblicdal allele is $f(2) > 0.5$; that is, the fitness of a parasitoid developing in a clutch of two eggs must only exceed half of the fitness of a parasitoid developing alone. Moreover, this condition holds also when the allele for tolerance is recessive. Thus, polyembryony, once established, substantially relaxes the conditions for the spread of a tolerant allele and may facilitate the transition from solitary to gregarious development, thereby allowing polyembryonic parasitoids to occupy a life history niche of small yet tolerant broods.

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cooperation between offspring. Soldier larvae benefit their clone-mates by eliminating competitors inside the host, and die prematurely. This extreme form of altruism, comparable to that found among eusocial insects, might have evolved because of the high relatedness between clone-mates (Gardner *et al.*, 2007). In several species, soldiers have been shown to attack according to relatedness, with higher levels of aggression expressed towards less related competitors (Giron *et al.*, 2004; Segoli *et al.*, 2009b). This further supports the idea that inclusive fitness plays an important role in this system.

The genetic conflict among offspring, and thus the advantages of reducing it, is expected to be especially strong within small broods (Box 3). In these broods, an individual may benefit highly by overcoming even a single sibling, whereas in larger broods the inclusive fitness from the many siblings may be large enough to compensate for the loss of individual direct fitness. This is in agreement with a genetic model of the evolution of solitary vs. gregarious development in parasitoids (Godfray, 1987). The model predicts that in small broods a siblicidal mutant is likely to invade and that only when clutch size is above a certain threshold will tolerance be evolutionarily stable. Further modelling demonstrates that factors that increase the average relatedness between offspring broaden the conditions under which a nonsiblicidal mutant can successfully invade a siblicidal population (Rosenheim, 1993). Because polyembryony produces clones of genetically identical offspring, once established, it may facilitate the transition from solitary (siblicidal) to gregarious (nonsiblicidal) development (Box 4).

Nevertheless, for the potential advantages of gregarious development to drive the initial transition from monoembryony to polyembryony, parasitoid offspring would need to be able to adjust their aggressive behaviour in response to the relatedness of other larvae within a shared host. Otherwise, offspring of a polyembryonic mutant would likely be as aggressive towards clonemates as other larvae, eliminating any immediate advantages of polyembryony, in spite of its potential future advantages. This is analogous to Frank's (1996) suggestion that reduction in symbiont genetic diversity cannot be favoured in hosts just by its long-term consequences on conflict reduction. Although there is vast evidence for kin-discrimination ability in different animal taxa (Sherman *et al.*, 1997), evidence for kin discrimination in monoembryonic parasitoids is equivocal (Fellowes, 1998). For example, female *Nasonia vitripennis* do not alter their sex ratios when competing for oviposition sites with siblings compared to unrelated wasps (Reece *et al.*, 2004; Shuker *et al.*, 2004). Evidence for kin discrimination includes avoidance of superparasitising hosts already parasitized by a relative (Marris *et al.*, 1996; Lize *et al.*, 2006) and increased efficiency of host utilization by gregarious parasitoids when sharing the host with siblings vs. nonsiblings (Hoffman, 2009). Whether kin recognition by parasitoids is exceptional or common, and whether larvae in lineages making a transition to polyembryony were able to discriminate between kin and nonkin remains an open question.

Based on the aforesaid, we infer that the role of polyembryony in reducing genetic conflict among offspring would have been most important at, or immediately following, the initial transition from

monoembryony to polyembryony, where the extent of embryonic division was probably restricted. It may still be important in those polyembryonic species where brood size is relatively small, for example in some polyembryonic platygasterids. The small host size in these species may restrict optimal brood size below the threshold that would normally allow for stable nonsiblicidal development. Reduced genetic conflict among siblings probably cannot explain the substantial increase in the extent of clonal division that has occurred in drynid and some encyrtid species.

Hypothesis III: brood-size adjustment

Craig *et al.* (1995, 1997) suggested that the main selective force favouring polyembryony is the inability of the parents to foresee the amount of resources that will be available for their young in the future. Under such circumstances, the parents may benefit from passing on some of the control over brood size to the offspring themselves. For example, a koinobiont parasitoid female attacking a host at a relatively early stage may not be able to foresee the host's future carrying capacity, because most of the host's growth occurs after parasitism and therefore, cannot match her clutch size to the eventual availability of host resources (Harvey, 2005). A similar argument was suggested to explain the inability of females to adjust sex ratio in their offspring to future resource availability (West & Sheldon, 2002). A possible solution may be to lay one or a few eggs, leaving the decision of how many offspring to produce from each egg (through polyembryony) to the offspring themselves. The offspring may better be able to assess host quality during their development and adjust proliferation levels accordingly. In spite of the attractiveness of this hypothesis, it has rarely been tested experimentally.

Indirect evidence for the importance of this hypothesis includes the substantial variation in brood size within polyembryonic species. In *C. floridanum* and *C. bakeri*, for example, brood size varies from 600 to over 3000. In *C. koehleri* it varies from 10 to 60, in *Macrocentrus grandii* from 10 to 25, etc. Moreover, there are several demonstrations that brood size is correlated with host mass or quality. For example in *C. floridanum*, broods within hosts that were starved for 48 h are about 50% smaller than those emerging from unstarved hosts (Giron *et al.*, 2004). In addition, clones emerging from hosts that were fed better quality food are larger than those emerging from hosts reared on poorer food (Beach & Todd, 1986; Lampert & Bowers, 2010). However, these variations in brood size may result from differential mortality during development rather than differential embryonic division, i.e. they may result from adjustment of secondary rather than primary brood size.

In previous studies, we tested the hypothesis of primary brood-size adjustment in *C. koehleri*. We found

that (i) embryonic division occurs at a relatively early stage of development (Segoli *et al.*, 2009a), thus, offspring still may not be able to foresee the final carrying capacity of the host at the time of proliferation; (ii) the body size of emerging wasps is negatively correlated with final brood size (Segoli *et al.*, 2009a,b), suggesting that the number of offspring is not fully adjusted to the host carrying capacity; (iii) host starvation during the proliferation phase has a minor, nonsignificant effect on the number of wasp embryos formed during development (as determined by dissecting hosts) and the number of wasp offspring emerging from the host (Segoli *et al.*, 2010); and (iv) host carrying capacity may be affected by brood size rather than the reverse, as parasitized hosts grow to reach larger sizes than unparasitized hosts, and hosts parasitized by two wasp clones are larger than those parasitized by one clone (Segoli *et al.*, 2009b). In the related species *C. bakeri*, Saeki *et al.* (2009) found indirect evidence for primary brood-size adjustments but concluded that most of the modification is achieved through manipulation of host growth. These combined results do not strongly support the hypothesis of polyembryony as a means of increasing offspring control of brood size. Nevertheless, further studies are required to test this possibility in other polyembryonic species and to explore the effect of other possibly important sources of variation in host quality on clone size.

Additional hypotheses

Additional factors, which were not included in our analyses, may select for polyembryony (Table 2). First, polyembryony may reduce the time costs of oviposition. A reduction in oviposition duration may increase the reproductive success of female parasitoids that are limited by the time available to locate suitable hosts (Rosenheim, 1999). Additionally, accelerated host handling may reduce the vulnerability of ovipositing females to disturbance or predation (Weisser *et al.*, 1994; Heimpel *et al.*, 1997; Barzman & Daane, 2001). If this was a main selective force, we would expect polyembryony to be more common where oviposition duration or the mortality risks during egg laying are elevated. However, most of the polyembryonic species parasitize the host egg. This probably involves less host handling and fewer associated risks than parasitizing larval or adult host stages, which can defend themselves (Gross, 1993). Another means of reducing the time costs of depositing large clutches may be achieved by laying multiple eggs in rapid succession during a single insertion of the ovipositor. For example, in the monoembryonic braconid wasp *Apanteles congregatus*, a female may lay up to 300 eggs in 2–3 s (Godfray, 1994). Thus, in at least some species, a reduction in the duration of oviposition may be achieved through an alternative mechanism that is much simpler than polyembryony. Nevertheless, the importance of time limitation in the evolution of polyembryony should

Table 2 Additional hypotheses for the evolution of polyembryony in parasitoid wasps.

Hypothesis	Assumptions	Predictions	Test of predictions	Relative importance
Overcome time limitation	Reproductive success of female parasitoids is limited by the time required to deposit eggs on suitable hosts. High mortality risks during oviposition	Oviposition duration is short for polyembryonic relative to monembryonic species. Poleymbryony more common when host handling time and risks during oviposition are higher	Most polyembryonic species are egg-larval, thus, the risks during oviposition stemming from host defences are relatively low	Probably not the main selective force. May provide an additional advantage
Escape host immune system		One or few eggs may escape host immune system, whereas a larger number of eggs may elicit a stronger defensive response from the host	Prediction not supported*	Not likely to be important
Overcome host volume limitation	Young hosts cannot carry a large number of parasitoid eggs, although they may accommodate many parasitoids at later stages	Combined volume of the number of eggs that is equivalent to brood size in polyembryonic wasps is large relative to the volume of the host	Volume of egg multiplied by brood size for *Copidosoma floridanum* is equivalent to 70% of host volume at parasitism†	Not likely to play a role during the initial transition to polyembryony and in most extant polyembryonic species

*Blumberg & Luck (1990) and Ode & Rosenheim (1998).
†Current paper.

be further explored. This may be achieved, for example, through comparison of oviposition durations of polyembryonic and monoembryonic species with broods of similar sizes.

Second, by laying only one or few eggs per host, the parasitoid's offspring may 'escape' detection by the host's immune system during the initial and highly vulnerable developmental stages, whereas a larger number of deposited eggs might elicit a stronger defensive response from the host. Empirical evidence, however, points in the opposite direction: larger clutches of eggs may better survive encapsulation responses of their hosts (Blumberg & Luck, 1990; Ode & Rosenheim, 1998), perhaps via overwhelming the host immune system (van Alphen & Visser, 1990).

Third, a young host may be physically unable to accommodate a large number of parasitoid eggs, even if it could potentially provide resources for a large number of parasitoid larvae once it has grown. For example, in the polyembryonic species *C. floridanum*, each wasp egg produces around 1200 individuals. The host egg's volume is approximately 50 000 000 μm^3, and the volume of the wasp egg is approximately 30 000 μm^3. It seems unlikely that the host egg could carry 1200 wasp eggs, which would be equivalent to approximately 70% of its volume. Polyembryony allows females to lay one or a few eggs per host, with gradual multiplication of offspring occurring later, after the volume of the developing host has increased. Volume limitation is more likely to occur when brood size is extremely large,

a condition that does not seem to be fulfilled in most polyembryonic species, and which is furthermore unlikely to characterize the transitional stage from monoembryony to polyembryony. Nevertheless, this hypothesis cannot be ruled out, and the volume constraint should be further explored. This can be carried out by further comparisons of initial host volume to the volume of the parasitoid egg multiplied by brood size.

Possible interactions

The aforementioned hypotheses are not mutually exclusive, and furthermore, they are likely to interact in several ways, increasing the potential adaptive value of polyembryony. For example, even in cases where brood size is adjusted secondarily (through embryonic or larval mortality) rather than primarily (through the number of embryonic divisions), polyembryony may confer an advantage. This is because it allows females to produce an excess of offspring (as insurance, or to allow later adjustments of brood size) while avoiding egg limitation. Producing an excess of offspring to ensure survival may be the only explanation for the enigmatic life history of *Macrocentrus ancylivorus*, where development is polyembryonic, but only one larva survives to emerge (Daniel, 1932). Brood-size adjustment may also interact positively with the reduction of genetic conflict. According to this view, polyembryony could be advantageous, because it reduces the conflict

between offspring throughout the process of brood-size reduction. Thus, polyembryony may allow adjustments of brood size while avoiding high costs of egg production and expensive competition between offspring in the struggle for survival.

Another factor that may create interactions between the selective forces is the risk of superparasitism or multiparasitism, i.e. oviposition in a host already parasitized by conspecific or heterospecific parasitoids, respectively (van Alphen & Visser 1990; Godfray, 1994). Polyembryony may confer an additional benefit if it increases the ability of some members of the clone to survive competition. One way in which this may be achieved is through an increase in the number of offspring per host (van Alphen & Visser 1990). In accordance with this hypothesis, females of the solitary monoembryonic parasitoid *Comperiella bifasciata* produce larger clutches when the perceived risk of competition is higher (Rosenheim & Hongkham, 1996). In contrast, mathematical models and empirical evidence show that gregarious parasitoids produce smaller clutches when the anticipated intensity of competition is high (reviewed in Godfray, 1994). This is because under the assumption of egg or time limitation, a female should invest less in low-quality parasitized hosts. Polyembryony may be adaptive, as it allows females to produce large broods enhancing the probability of offspring survival, without paying these associated costs. Additionally, polyembryony may induce cooperation between siblings against competitors. Indeed, it was shown that the allocation to the soldier caste within polyembryonic clones increases in response to interspecific competition (Harvey *et al.*, 2000). Thus, the risks of superparasitism and multiparasitism may select for polyembryony through an interaction of several selective forces: it may allow offspring to adjust their numbers to available resources in the case of competition, and it may increase survival of offspring by elevating their numbers while avoiding egg (or time) limitation, or by promoting cooperation against competitors.

Constraints on the evolution of polyembryony

Our analysis demonstrates that polyembryony may have substantial advantages under a wide range of circumstances. Why, then, is it not more common than observed? One possibility is that it is indeed more common than so far documented, but characterizing polyembryonic development involves detailed investigations of embryogenesis that have yet to be accomplished for many parasitoid species (Parker, 1931; Strand, 2003). Nevertheless, it cannot be ruled out that even in cases where polyembryony would be adaptive it has not always evolved, simply because it is difficult to 'invent'. Indeed, polyembryonic development was shown to

represent a major departure from typical insect embryogenesis, as it involves features such as: holoblastic cleavage, the formation of an extraembryonic membrane, the introduction of a proliferative phase, and the *de novo* establishment of embryonic axes (Grbic *et al.*, 1998; Grbic, 2003; Zhurov *et al.*, 2007). Thus, there may be major developmental constraints on the evolution of polyembryony, preventing it from becoming a more common strategy.

Future work

The brood-size adjustment hypothesis may be tested directly through studying the effect of different manipulations of host quality on primary and secondary brood size in additional polyembryonic wasps. Evidence for primary adjustments will support the importance of this hypothesis, whereas evidence for secondary adjustments may instead suggest interactions with the other selective hypotheses. The other two hypotheses concern evolutionary processes and thus are difficult to test directly. Further progress would be facilitated by understanding more clearly the life history traits of the ancestral lineages within which polyembryony evolved (e.g. was the ancestor a solitary or gregarious parasitoid? what size clutches were produced? what was the size of the host? was it able to discriminate between kin and nonkin?). Reconstruction of the phylogenies of polyembryonic species and their monoembryonic sister taxa would be extremely helpful in this regard. For example, if polyembryony evolved within solitary parasitoid lineages, this would underscore the importance of the relaxation of genetic conflict in promoting the transition from solitary to gregarious development. Polyembryonic braconids of the genus *Macrocentrus* may have evolved from a solitary parasitoid ancestor, as all known monoembryonic species in this genus appear to be solitary (M. Sharkey, pers. comm.). A gregarious ancestor would underscore the importance of overcoming egg limitation, especially if brood size increased with the transition to polyembryony. This may be the case in polyembryonic encyrtids (e.g. *Copidosoma*), which are probably derived from a group where gregarious development was the norm (J. Noyes, pers. comm.), and produce the largest known broods (Alvarez, 1997). Further indications for the importance of egg limitation would be an evolutionary increase in host size and brood size or an evolutionary decrease in parasitoid body size or fecundity.

Conclusions

We suggest that polyembryony allows parasitoid species to occupy life history 'space' in ways that monoembryonic species cannot, by allowing them: (i) to produce large brood sizes (per host) while avoiding egg limitation; (ii) to produce small broods without being invaded by

siblicidal (or otherwise competitive) mutants; and (iii) to adjust offspring number to host carrying capacity. Reducing the genetic conflict between offspring may have been particularly important during, or immediately following, the initial transition from monoembryony to polyembryony, and in those species where brood size remains relatively small, whereas reducing the cost of egg production may be more important in maintaining and accelerating the degree of embryonic division in species with larger broods. Empirical data provide little support for the brood-size adjustment hypothesis, although it is likely to interact with other selective forces favouring polyembryony.

Acknowledgments

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