

The ‘paradox’ of polyembryony: A review of the cases and a hypothesis for its evolution

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Summary

Animal polyembryony appears to be paradoxical because it clones an unproven genotype at the expense of genetic diversity in a clutch. However, it is employed by at least 18 taxa in six phyla (excluding instances of occasional twinning). Most polyembryony occurs in parasitic stages or in other environments whose quality is not predictable by the mother; in some instances, it compensates for a constraint on zygote number. We predict that polyembryony is likely to evolve when the offspring has more information regarding optimal clutch size than the parents.

Keywords: asexual reproduction; clonal reproduction; clutch size; environmental predictability; life history evolution; parasites; parasitoids; polyembryony

How quaint the ways of Paradox!
At common sense she gaily mocks!
(W.S. Gilbert, 1879, *The Pirates of Penzance*).

Introduction

The term ‘polyembryony’ refers to the splitting of one sexually produced embryo into many. The resultant offspring are genetically identical to each other but different from their mother. In this way, polyembryony differs from various forms of budding or vegetative reproduction, in which adult organisms produce genetic replicas of themselves. It differs from ordinary sexual reproduction in that a newly formed zygote divides to form many siblings of the same genotype.

The vast majority of organisms produce all their zygotes sexually. The selective value of sex (i.e. recombination) may be that it provides enough genetic variation among offspring to face expected environmental variation (Williams, 1966). Parthenogenesis is beneficial in some circumstances because it allows rapid replication of the mother’s relatively successful genotype. If the environment does not change very quickly, parthenogenesis (or any other type of asexual reproduction) may be advantageous compared with sexual reproduction. In many aphids, certain other insects, some worms and cladocerans, parthenogenesis continues until severe environmental changes begin, at which point sexuality intervenes (Slobodkin, 1980).

Polyembryony, however, appears to lack the advantages of both sexual and asexual reproduction. Unlike parthenogenesis, which produces many replicates of a proven genetic winner, the novel genotype of polyembryonic offspring differs from that of their mother. Unlike sexual re-

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production, which produces a full spectrum of genotypes to meet a variety of environmental conditions, polyembryony ‘bets’ on only one unproven genotype.

The paradox of polyembryony is that organisms are committing an apparent error equivalent to that of purchasing multiple tickets of the same number in a lottery, even though there is no reason to prefer one number over another (Williams, 1975). The ‘paradox’ can be resolved by finding an advantage to polyembryony which counterbalances the ‘same number lottery ticket’ disadvantage. Polyembryony occurs in the life cycle of a surprising diversity of animals, and more cases will almost surely be found. In this paper, we propose a number of environmental conditions that would seem to favour polyembryony. Most of these involve situations where offspring are more able than their mothers to predict the appropriate clutch size (Craig *et al.*, 1995).

Definition

Before embarking on an analysis of why polyembryony exists, it is useful to consider the range of phenomena to which the term has been applied. The most restrictive definition of polyembryony refers to embryonic cloning, the physical splitting of cells after the first few mitotic divisions into separate embryos. Following Bosch *et al.* (1989) and Moore (1981), for example, we extend the term polyembryony to cases where a post-embryonic, but pre-adult, individual clones itself. To our knowledge, such cases are limited to organisms with complex life cycles where the intermediate life-history phase, or larva, occupies an environment that is quite different from that of the adult. We will include these cases in our discussion, since the intermediate stage represents a genotype that is still unproven with respect to the adult life cycle. In contrast, it is universally agreed that asexual reproduction in a sexually mature adult, such as budding or fission, is not polyembryony.

In all cases that meet our definition, offspring are genetically identical to each other, but distinct from their parent(s). Inherent in the existence of embryonic or other pre-adult cloning is the prior existence of a meiotic product, the egg. In some cases, such as the male progeny of polyembryonic parasitoid wasps, this egg is not fertilized to form a diploid zygote. Yet because it is the product of meiosis, it is genetically distinct from the parent. This is in sharp contrast to most adult cloning, which involves no intervening meiosis, and results in individuals that are genetically identical to the parent. Thus with polyembryony, the genet at the time of cloning includes no individuals that have reached reproductive maturity, while in the more common cases of adult cloning, the genet always includes an adult.

There are, however, many processes which have been referred to as polyembryony by some, but do not meet our criteria. These fall into three general categories: (1) parthenogenetic or asexual multiplication by juveniles (which live in the same environment as the adults); (2) early onset of coloniality; and (3) accidental (sporadic) twinning. Examples are listed in Appendix 2.

Animals that use polyembryony

Polyembryony as we have defined it occurs in a wide variety of unrelated organisms, including four unrelated families of parasitoid wasps, one order of bryozoans, some parasitic flatworms and hydrozoans, and armadillos (Table 1). We review the natural history of each of these cases in Appendix 1.

Based on its phylogenetic distribution, polyembryony seems to have evolved on at least 15 separate occasions within six animal phyla (see Table 1). This figure is probably an underestimate, as biologists have examined only a tiny fraction of the Earth’s biota in sufficient detail to detect polyembryony. Of the known cases, eight involve cloning embryos either immediately following fertilization or in blastula-like aggregates of cells. The remaining 10 cases involve cloning in

Table 1. Distribution of polyembryony in the animal kingdom

Phylum	Class	Order	Representative species	Clones at:	Life habit	References
Cnidaria	Hydrozoa	Trachylina	<i>Pegantia</i> spp.	Larva	Endoparasitic	Shostak (1993), Bigelow (1909)
			<i>Cunina</i> spp.	Larva	Endoparasitic	Shostak (1993), Bigelow (1909), Berrill (1949)
		Hydroida	<i>Cunocantha</i> spp.	Larva	Endoparasitic?	Bigelow (1909)
			<i>Polypodium hydriforme</i>	Larva	Endoparasitic	Raikova (1973, 1980)
Platyhelminthes	Monogenea	Gyrodactyloidea	<i>Gyrodactylus elegans</i>	Embryo	Ectoparasitic	Katheriner (1904)
		Eucestoda	<i>Echinococcus</i> spp.	Larva	Endoparasitic	Noble <i>et al.</i> (1989)
	Trematoda	(subclass)				
		Digenea (subclass)	<i>Schistosoma</i> spp.	Larva	Endoparasitic	Noble <i>et al.</i> (1989)
Arthropoda	Cirripedia (Crustacea) Insecta	Rhizocephala	<i>Loxothylacus panopaei</i>	Larva	Endoparasitic	Glenner and Høeg (1995)
		Hymenoptera				
		f. Encyrtidae	<i>Copidosoma</i> spp.	Embryo	Endoparasitic	Strand (1989a,b,c), Ode and Strand (1995)
		f. Platygasteridae	<i>Platygaster</i> spp.	Embryo	Endoparasitic	Godfray (1994)
Bryozoa	Stenolaemata	f. Braconidae	<i>Macrocentrus</i> spp.	Embryo	Endoparasitic	Godfray (1994)
		f. Dryinidae	<i>Aphelopus theliae</i>	Embryo	Endoparasitic	Godfray (1994)
		Strepsiptera	<i>Halictoxenos simplicis</i>	Embryo	Endoparasitic	Jeannel (1951)
		Cyclostomata	All species studied to date (e.g. <i>Crisia</i> spp.)	Embryo	Free-living (brood chamber)	Borg (1926, 1933), Calvet (1900), Harmer (1890, 1893)
Echinodermata	Asteroidea	Paxillosoida	<i>Luidia</i> sp.	Larva	Free-living	Jaekle (1994), Bosch <i>et al.</i> (1989)
		Unidentified non-paxillosoid		Larva	Free-living	Jaekle (1994), Bosch <i>et al.</i> (1989)
	Ophiuroidea	Ophiurida	<i>Ophiopluteus opulentus</i>	Larva	Free-living	Mortensen (1921)
Chordata	Mammalia	Edentata	<i>Dasypus</i> spp.	Embryo	Free-living (uterus)	Galbreath (1985)
		f. Dasypodidae				

intermediate life-history phases. All polyembryonic stages seem to be able to take up exogenous nutrients.

It is striking that 13 of the 18 cases involve organisms with parasitic, and particularly endoparasitic, lifestyles (Table 1). We interpret the existence of polyembryony in these cases to be related to the mother's inability to predict optimal brood sizes, rather than a general numerical advantage to cloning in parasites. The remaining cases are not united by obvious features, suggesting that a number of unrelated factors may favour polyembryony: (1) polyembryony may provide a means of taking advantage of a necessarily prolonged larval phase; (2) polyembryony may produce many offspring even when sperm are limiting; and (3) constraints on the number of offspring or eggs an individual can produce (due, for example, to phylogenetic history) can be circumvented by polyembryony. All four hypothesized factors are discussed below.

Why reproduce via polyembryony?

There are many taxa in which polyembryony appears to be a normal, efficient and, in many cases, obligate mode of reproduction (Table 1). Because polyembryony is not restricted by taxonomic group or developmental mode, the adaptive value and resolution of the paradox must, at least in part, lie with ecological conditions. We start by discussing how polyembryony can fit into the classical issue of size versus number of offspring, and then suggest environmental circumstances which could outweigh the disadvantage of genetic monotony. The predictions of these (ecological) hypotheses can then be compared with field observations to see if polyembryony occurs when, and only when, the stated ecological conditions are found.

Size versus number of offspring

If there is a fixed assignment of resources to reproduction, it is impossible to increase both the size and number of young simultaneously (e.g. Lack, 1947; Strathmann, 1985). The outcome of this trade-off may depend on the mother's physiological condition – obviously within taxonomic boundaries (oysters produce millions of tiny, inexpensive eggs, whereas mammals tend to have few costly young). For instance, better fed *Daphnia* produce smaller, more numerous eggs than less well fed *Daphnia* (Slobodkin, 1954). Small size may be advantageous when food is sufficiently abundant, while larger, more yolk-filled embryos may have a higher relative survival rate when food is in short supply (Charnov *et al.*, 1995).

We are accustomed to seeing selection for smaller and more numerous offspring expressed in a larger number of zygotes formed by the mother. But what if this goal was achieved by another path, such as polyembryony? A selective force for a particular phenotype need not always manifest itself in the same ontogenetic pathway (e.g. Wray and Raff, 1991). From this perspective, polyembryony represents a special case of the general problem of choosing between a large number of small young and a smaller number of larger young, *but relegating that choice to the initial embryo rather than its mother*. In certain situations, offspring may simply be in a better position to determine optimal clutch size. We review these situations below.

Ecological conditions favouring polyembryony

Availability of information to parasites. An embryo might be able to judge the optimal compromise between resources per embryo and number of embryos better than its mother when its environment is sealed off from that of the mother (Godfray, 1994, pp. 118–119). This may be particularly true in parasites, whose offspring develop within the body cavity of a host, be it a moth egg (parasitoid wasps), a fish egg (endoparasitic hydrozoan), or an adult snail or vertebrate host (endoparasitic

flatworms; see Appendix 1). Host size and health will vary considerably, and the parasite thus has to optimize brood-size based on these factors. For example, the number of successfully developing parasitoids that can emerge from a single host may not be apparent to the maternal *Copidosoma floridanum* wasp at the time of egg-laying. Depositing too many eggs inside the host may lead to increased mortality and smaller size upon emergence due to competition for limited resources. In contrast, if there are too few offspring within the host, the parasitoids are apparently not numerous enough to eat their way out, and all of them will die (Ode and Strand, 1995). Most parasitoid wasps remain monoembryonic, however, which may suggest that host shifts or changes in host development may have favoured the evolution of polyembryony in only some parasite taxa.

Among taeniid tapeworms, *Echinococcus* spp. have the largest potential for polyembryony and the widest range of intermediate hosts in which budding occurs (from rabbits to moose; Moore, 1981). This illustrates what we expect to find: polyembryony among parasites and parasitoids whose hosts' quality is highly variable and difficult to assess.

Parasitoid wasps are to some extent capable of assessing host quality both visually and with taste receptors on their ovipositor (Hardy *et al.*, 1993; Godfray, 1994). However, the use of polyembryony may represent an alternative solution to this problem, by leaving the evaluation of the resource to the offspring. The evolution of polyembryony might therefore relax selection for the ability to test the host prior to oviposition. In addition, the polyembryonic stages of tapeworms, digeneans and rhizocephalan barnacles (Appendix 1) arise after dispersing away from their mother, making it impossible for the mother to predict the size, quality and infestation load of the offsprings' hosts.

In polyembryonic parasites, the evolution of 'virulence' must also be controlled at the progeny stage. Although parasitoid wasps (by definition) completely exhaust their host, they appear to do so only at the very final host instar, thus optimizing the number of parasitoids which can be produced. Trematode (and other parasite) larval stages also live in unlimited resources only while their host is alive (Baer and Joyeux, 1961, p. 595), and may depend on the mobility of an intermediate host for transmission to the final host. Parasitic larvae must therefore avoid killing their intermediate host by over-multiplication. Again, the optimal amount of polyembryonic budding is best evaluated from inside each individual host.

Availability of information to feeding larvae. Polyembryony may be favourable for teleplanic larvae, which traverse whole oceans lacking appropriate habitats for metamorphosis. These animals must remain in the plankton for a long time to disperse to new continents. By budding new larvae off its larval arms polyembryonically, a starfish brachiolaria may be able to prolong its time until metamorphosis. Without this delay, it is likely that the larvae would settle in an inappropriate habitat (the benthos below the mid-ocean gyres is dominated by muddy, nutrient-poor substrates) and die.

In addition, if planktonic resources are abundant, polyembryonic larvae could use these excess resources to reproduce asexually, increasing the fitness of the genet. A few starfish larvae appear to do this, as do the alpha larvae of *Cunina proboscidea* and other narcomedusan hydrozoans.

Environmental variability, sperm limitation and lengthy oogenesis. Polyembryonic reproduction could be an important, flexible adaptation in brooding females, if sperm are limiting. This would enable an adult to produce many offspring, even if only a single egg was fertilized. This would permit a response to increasing food availability, even when the production of numerous sexual offspring is not an option. Marine free-spawners may often be sperm-limited, because sperm are diluted rapidly in the water column (Levitan, 1995; Levitan and Petersen, 1995). Polyembryony could also be advantageous if females cannot store sperm, and cannot easily find mates (as, for example, in obligatorily out-crossing parasites).

Polyembryony may also allow species with lengthy oogenesis to produce more offspring quickly. This may be advantageous in cases where food abundance changes dramatically in a short time period. Cyclostome bryozoans appear to have a long oogenesis period, may be sperm-limited (Ryland, 1996), and their food source, phytoplankton, is extremely patchy. Polyembryony would enable the adult to produce more offspring when and if its catch of plankton suddenly increased. Both cyclostome bryozoans and the parasitic flatworm *Gyrodactylus* appear able to transfer nutrients to their brood-chamber at any time following fertilization, and hence may be extremely flexible in their clutch size, due to polyembryony.

Constraints on offspring production. It may be impossible for the parent to lay enough eggs to take advantage of the local environment. Polyembryonic parasitoid wasps are among the smallest parasitoids (Godfray, 1994) and may, therefore, be constrained in the number of eggs which they can lay. Nonetheless, they have the largest clutch sizes of all parasitoids (Godfray, 1994). It is difficult to imagine a tiny wasp, such as *Copidosoma floridanum*, producing more than 600 eggs at once – the minimum number necessary to consume the caterpillar host and hatch out successfully (Ode and Strand, 1995).

Polyembryony never occurs in birds or reptiles, perhaps because testing of the external world for abundance of resources cannot be done from within a hard shell. If double yolks ever produce twin chicks, we have not heard of it. By the same argument, we would not expect polyembryony among mammals. The mother, by holding the young *in utero*, is in a better position to assess environmental quality than the young themselves. Apart from the rare ‘accident’ of identical twins in mammals (see Appendix 2), polyembryony is not found in vertebrates, with the exception of armadillos. While reproduction in armadillos has been termed polyembryony (Galbreath, 1985), we contend that multiple embryos arising from a single egg in the armadillos is a fundamentally different process than the other cases mentioned above. Specifically, although double ovulation has been observed in *Dasypus novemcinctus*, no-one has ever found a case where more than one embryo has implanted in the uterus. This may be explained by the unusual, kite-shaped uterus in *D. novemcinctus*, which appears to have only one blastocyst implantation site. Two of the three extant genera most closely related to *Dasypus* (*Tolypeutes* and *Cabassous*) produce only one offspring, supporting the idea that ancestral *Dasypus* had a typical litter size of one (Wetzel, 1985). Galbreath (1985) has therefore suggested that polyembryony has evolved in this genus because it is the only way to produce a larger clutch size given the constraint of a single implantation site.

How does polyembryony evolve?

Despite the advantages outlined above, polyembryony is a relatively rare life-history strategy (Hardy, 1995a). This could be because it is somehow ‘difficult’ to evolve. This question cannot be addressed directly with the data at hand and clearly deserves further attention. Two lines of indirect evidence, however, suggest that the origin of polyembryony is probably not a particularly ‘difficult’ evolutionary transition, at least in many clades. The first is that accidental polyembryony, in the form of monozygotic twinning, is not a rare phenomenon in many taxa (Appendix 2). The second line of evidence comes from experimental embryology. Direct manipulations indicate that single blastomeres in the very early embryos of many species are able to develop into adults. Examples of taxa where this is possible include some cnidarians, nemerteans, platyhelminths, molluscs, bryozoans, phoronids, echinoderms, cephalochordates and vertebrates (Willadsen, 1979; Gilbert, 1991). Although direct proof that the resulting individuals are capable of sexual reproduction is lacking in some of the cases, these experiments do demonstrate that artificial polyembryony can generate individuals that appear phenotypically normal.

In some cases, however, polyembryony involves highly derived developmental processes that do not seem easily generated from related species that lack this trait. In cyclostome bryozoans and the wasp *Copidosoma*, cloning occurs by fission of irregularly shaped 'blastulae' that are composed of many cells. These are not simple cases of cells falling apart after the first mitotic division (see also the strepsipteran mechanism in Appendix 1). In *Copidosoma*, detailed studies of morphology and gene expression indicate a substantive departure from the usual course of pterygote insect development (M. Grbic and M.R. Strand, personal communication). In seastars that clone as larvae, early morphogenesis is also very derived; cloned larvae often form guts and coeloms in ways that are strikingly different from the primary larva (Jaekle, 1994). It is difficult to imagine these highly modified modes of development as having arisen in a single step. Some of these derived features may, however, represent developmental modifications that are not central to achieving polyembryony, but are secondary adaptations to a polyembryonic life history.

Why don't all organisms reproduce polyembryonically?

If our arguments are correct, conditions favouring the evolution of polyembryony should be present in a great number of animals. So why isn't polyembryony more common? Is it that many cases of asexual reproduction in larvae or embryos are yet to be discovered? Most cases of polyembryony have been revealed either by skewed sex ratios, resulting from the production of a clutch of all one sex (e.g. parasitoid wasps), or by unusually high fecundity. None of the cases listed above were discovered by genetically typing the offspring, a method which may well reveal other cases, as well as lead to a much greater understanding of the evolution of polyembryony.

Polyembryony does not seem to occur in dry environments in which the embryo has to be sealed off by a tough chorion (as in bird and insect eggs). A chorion prevents the offspring from taking up nutrients as well as from increasing their total volume. We postulate that most organisms that do not have these developmental constraints reproduce monoembryonically because the advantages of genetic heterogeneity among the young in a litter overshadow the numerical rewards of polyembryony. In most animals, producing a large number of (small) zygotes initially does not seem very costly or constrained – sperm are cheap. However, because many organisms with polyembryony, such as parasitoid wasps, produce numerous clutches, the genetic diversity produced could be just as great as that in monoembryonic organisms, with the important difference that all genetic variability would be spread among clutches (Hardy, 1995b).

Polyembryony is the result of a compromise between the mother's genes and those of the offspring (and father). The genetic composition of polyembryonic offspring differs from that of the parents, so that these perspectives may be in conflict (Haig, 1993), particularly in organisms which brood their offspring (e.g. cyclostome bryozoans and armadillos). While the mother's genes might favour producing a genetically diverse clutch, each offspring might benefit from increasing its own representation in the brood chamber at the expense of other genotypes. However, polyembryonic splitting during early cleavages may actually still be controlled by maternal mRNAs, and only later governed by the offsprings' own genes. Hence the timing of gene action relative to the timing of cloning may be critical in determining which party (mother or offspring) 'wins' the conflict of interest.

Because parasitic polyembryonic individuals are genetically identical, they are more likely to cooperate with one another inside the host than a genetically diverse clutch (Grbic *et al.*, 1992). Certain attributes of population structure, such as inbreeding or haplo-diploid sex determination, however, result in a higher relatedness between mother and offspring, reducing the genetic conflicts between them (e.g. Hardy, 1995b). Hence kin selection may enhance the likelihood of the evolution of polyembryony by making this mode of reproduction more similar to asexual reproduction via fragmentation, budding, etc. Conversely, polyembryony can contribute to an inbred population

structure: intermediate hosts of tapeworms commonly contain an immense number of polyembryonically multiplied larvae. The final host is likely to ingest many of these larvae when preying on the intermediate host and consequently will harbour a large number of genetically identical tapeworm adults (Moore, 1981). Not much genetic diversity will be lost by cloning embryos in this case, because genetically identical adults are likely to interbreed.

In addition to genetic costs, there are likely to be ecological trade-offs in polyembryonic reproduction. Starfish which bud larval arms polyembryonically extend their time in the plankton, and thereby increase their chances of mortality due to predators, starvation, etc. For many starfish (and other organisms), this trade-off may not be worthwhile, and therefore polyembryony might be restricted to teleplanic larvae with few other options.

Conclusions

The conditions that favour the evolution of polyembryony may be more diverse than previously suspected. Nonetheless, polyembryony is not to be universally expected due to the cost of low genetic diversity. Any time that polyembryony is found we expect our conditions to be met: either that the parent is particularly ill-equipped to sense the ecological circumstances of the young, or is constrained to produce much fewer eggs than a fortunate environment will bear (or both).

We do not believe that all cases of polyembryony have been discovered. While it might be objected that the purity of our hypothesis is compromised by our *a priori* knowledge of natural history, this objection cannot be valid if further investigations are undertaken whose results could not be known to us in advance.

We hope that our discussion of polyembryony will encourage further investigations of the timing and conditions of reproductive events. If there exists a case of polyembryony in which parental information about the environment of the embryos is as good as that of the embryos themselves, and the parents also have a high and flexible fecundity, then our analysis is incomplete and the paradox persists.

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Appendix 1: Organisms which use polyembryony

Hydrozoan cnidarians

Because planktonic medusae reproduce sexually, it is possible to view this stage as the ‘adult’, in which case both the planula larva that settles to the benthos and the feeding hydroid colonies which the planula produces could be termed ‘larvae’. Since many of these colonies then bud off genetically identical medusae, hydrozoans in general can be called polyembryonic (F. Boero, pers. comm.). In fact, since hydroid colonies often regress during unfavourable seasons, and regenerate anew in the spring (Arillo *et al.*, 1989; Boero *et al.*, 1992), when they resume budding new medusae, polyembryony may be a continual condition of hydrozoans. Nevertheless, since terming hydroid colonies ‘larvae’ is controversial at best, we have not included Hydrozoa as a group in Table 1, and will restrict our discussion below to a few remarkable cases. However, the diverse hydrozoan life cycles will undoubtedly yield more examples of polyembryony and are clearly deserving of further attention (Boero *et al.*, 1992; Boero, 1994).

Narcomedusan hydrozoans have a life cycle in which sexual and polyembryonic reproduction alternates. In *Peganthia smaragdina*, elongate tentaculate larvae bud (polyembryonically) within the gastric cavity of their parent, and are subsequently liberated as medusae (Bigelow, 1909). Bigelow (1909) also found several budding stolons of unidentified species of *Cunina* attached either to the bell or the gut of other species of jellyfish. It is unclear, however, whether these isolated cases represent cases of parasitism (but see Noble *et al.*, 1989, p. 431; Shostak, 1993).

Berrill (1949) reviewed other examples of embryonic budding in narcomedusan hydrozoans, including the extraordinary life history of *Cunina proboscidea*. In *C. proboscidea*, large female medusae produce eggs which can develop with or without fertilization. The fertilized eggs give rise to ‘gamma’ larvae, which metamorphose into dwarf male medusae. The unfertilized eggs – presumed by Bell (1982) to be diploid – develop into ‘alpha’ larvae, which through budding (polyembryony) generate ‘beta’ larvae. Both of these larval types (alpha and beta) develop into dwarf female medusae. Alpha medusae then produce eggs which develop (without fertilization) into large female medusae. Hence adult female medusae are produced by a series of asexual processes, involving budding (polyembryony) and apomixis. Beta medusae produce eggs which, when fertilized by gamma males, develop into large male medusae. Large male medusae are thus the product of a cycle which involves two episodes of meiosis followed by syngamy (Bell, 1982).

In addition to marine hydrozoans with polyembryony, there is a tiny freshwater hydrozoan, *Polypodium hydriforme* (Narcomedusae, Polypodiidae), first discovered in Eastern Europe as a parasite inside freshwater fish eggs, which has polyembryony (Raikova, 1973, 1980). Eighty percent

of the European sterlet (*Acipenser ruthenus*) and 20% of sturgeon (*A. guldenstadti*) in the major rivers of the former Soviet Union are infested with this species. It may be the only endoparasitic coelenterate. The free-living stages correspond to medusae in other narcomedusans, while the endoparasitic stage is homologous to the polyp phase (Bouillon, 1985).

The development of the larval *Polypodium*, like that of the parasitoid wasp *Copidosoma*, is closely coordinated with that of its host. A binucleate, single-cell stage is found in immature sterlet oocytes. By the time the host oocyte has started to accumulate yolk, the *Polypodium* is a two-layered planula approximately 1 mm long. It has a flagellated external layer, which eventually becomes the endoderm, and an internal layer of ultimate ectoderm. Both of these layers are surrounded by a capsule that serves as a digestive organ for consuming yolk (Raikova, 1980).

After a month, the planula develops into a stolon with buds which contain internally directed tentacles. At this stage, the *Polypodium* is a colony containing as many as a dozen of these buds. Each bud develops two indentations, and these indentations each become 12 tentacles which project into the stolon itself. As the fish eggs ripen and are released from the adult, the tentacles evert through a slit in the stolon. Simultaneously, the buds invert, developing a coelenteron lined by what had been the surface (exposed to the egg yolk). Polyembryony occurs in these organisms when the stolon breaks apart. Each bud breaks off to form a somewhat bifurcated polyp with 12 tentacles and a coelenteron full of fish egg yolk (see fig. 5.11c in Slobodkin and Bossert, 1991). These free living polyps subdivide by longitudinal fission, and crawl along the bottom using their tentacles as walking legs. Both sexes are then thought to crawl onto the sturgeon and place their gametes on the exterior.

Ectoparasitic flatworms

Some flukes (Cl. Trematoda) are ectoparasites of fish and occasionally of crustaceans. Polyembryony is best known and most striking in one species of these ectoparasites, *Gyrodactylus elegans*. In this species, found on the scales and fins of both marine and freshwater fish, two hermaphroditic worms mate by reciprocal transfer of spermatozoa. A single yolkless egg then grows and divides within the uterus, nourished through a thin wall separating it from the mother's gut. Unequal divisions produce a second embryo (which develops inside the first), a third embryo (which develops inside the second) and a fourth embryo (which develops inside the third), like a series of Russian dolls. The second (grand-daughter) embryo does not complete its development until the first (daughter) embryo is released from the parent (Katheriner, 1904). The newly released daughter need not reach the size of its mother before giving birth to the grand-daughter, however. Hence the production of offspring is accelerated. In this way, a single worm can give rise to as many as 2453 descendants in 30 days (Baer and Euzet, 1961).

Endoparasitic flatworms

Many endoparasitic digenetic flukes (e.g. schistosomes) multiply inside the intermediate host at the sporocyst stage (the first stage that hatches from the sexually produced egg) and at the next (redia) stage. Both the dynamics over time and the total number of cercariae produced this way can vary, even within trematode species; for example, with physiological state and species identity of the intermediate host (Esch and Fernández, 1993, p. 132).

Cestode larvae, most notably many taeniids, bud by several diverse mechanisms in the intermediate host (Moore, 1981). In the tapeworm *Echinococcus granulosus*, a large number of brood capsules are budded within the hydatid cyst, which forms in the intermediate host. Many scolices bud off in each brood capsule, resulting in up to a million protoscolices in a single cyst (Esch and

Fernández, 1993, p. 124). The scolices eventually infest the definitive host when it preys on the intermediate host (Noble *et al.*, 1989, p. 250).

Rhizocephalan barnacles

The Rhizocephala are a parasitic order of barnacles (Cirripedia, Crustacea) with a highly modified life cycle (Høeg, 1995). The sexually produced, pelagic cyprid larva does not, as in other barnacles, metamorphose into a sessile adult, but forms a 'kentrogon' after settling on a (decapod crustacean) host. The kentrogon then injects de-differentiated cells into the host body, which develop first into endoparasitic 'internae' until a female reproductive body emerges on the surface of the host. Dwarf males originate when cyprids settle on the externa.

Glenner and Høeg (1995) recently described the (polyembryonic) host invasion by the kentrogon's inoculation. In *Loxothylacus panopaei*, the injected primordial parasite is a motile, vermiform stage that splits into about 25 cells that move about independently in the host's haemolymph. It is not yet known whether all of them grow into internae; however, usually only one (rarely a few) of the internae will succeed to form a large, sexual externa. It is therefore not clear whether the polyembryonic multiplication after the cyprid stage will result in higher fecundity of the genet. Multiplied host invasion stages may be advantageous for manipulating the host's physiology, for locating the optimal site for externa-formation, or for competition with unrelated internae resulting from infection by a separate kentrogon.

*Parasitoid wasps**

Polyembryony was first described in the chalcidoid family Encyrtidae, where it is found in several closely related genera (tribe Copidosomatini) of egg-larval parasitoids of lepidopterans (Marchal, 1898). The best known life history of any polyembryonic species is that of the encyrtid wasp *Copidosoma floridanum* (Strand, 1989a,b,c).

In *C. floridanum*, a female inserts either one or two eggs into its host, the egg of a noctuid moth. Following deposition of the wasp egg(s), the host (moth egg) hatches into a larva, and develops up to its final instar without evident deleterious effects from the parasitoids inside it. Meanwhile, the parasitoid egg(s) divide, and a few (5–8 in *C. floridanum*) of these embryos develop rapidly, becoming 'soldiers' with large mandibles. These soldiers patrol the interior of the developing moth larva and prevent other parasitoids from invading the same host (Silvestri, 1906; Cruz, 1981, 1986). The soldiers do not feed, however, and die before their clone mates complete their development (Hughes, 1989). Although the mechanism which allows only a few embryos to develop precociously into soldiers is unknown, artificial elevation of the host's juvenile hormone titre or starvation of the host increases the number of defensive larvae produced (Godfray, 1994).

Development of the rest of the polyembryonic clone is also triggered by changes in the host's hormone titre (Strand *et al.*, 1990, 1991a,b), and occurs during the host's final instar. At this time, up to 1500 individual wasps (3000 in a related species: see Godfray, 1994) eat their way out of the host and pupate on the skin of the expired caterpillar. In addition to encyrtid wasps, polyembryony can also be found among members of three other, unrelated families of parasitoid wasps (e.g. Nénon, 1983), including the Platygasteridae, the Braconidae (Voukassovitch, 1927; Parker, 1931) and the Dryinidae (Ivanova-Kasas, 1972). Hence this trait appears to have evolved repeatedly within the Hymenoptera. Polyembryonic wasps are among the smallest of all parasitoids (Godfray, 1994).

*A recent excellent summary of parasitoids includes most of the polyembryonic hymenoptera (Godfray, 1994). Facts not otherwise attributed are to be understood as coming from Godfray's text.

Strepsiptera

The Strepsiptera are a holometabolous order of insects that parasitize Hymenoptera and Homoptera. Polyembryony has been described in one strepsipteran species which parasitizes halictid bees: *Halictoxenos simplicis* (Noskiewicz and Poluszynski, 1935). The highly modified endoparasitic females are wingless but get fertilized by winged males while they are inside the host (Jeannel, 1951, p. 1297). The female's abdomen, bathing in the host's haemocoel, contains thousands of zygotes, each of which is accompanied by vitelline cells. These cells migrate in between blastomeres and separate them, resulting in 2–40 polyembryonic offspring per zygote. Each embryo then develops into a 'triongulid' larva, which leaves the host, and is transmitted by hopping onto a new host bee which visits the flower or nest on which they occur.

Cyclostome bryozoans

Polyembryony is thought to occur in all living stenolaemate bryozoans (Ryland, 1970). Stenolaemate bryozoans are an ancient group, dating back to the Ordovician. Four of the five known orders became extinct at the Permian–Triassic boundary, leaving only one extant order, the Cyclostomata. Cyclostomes form colonies with one or a few common (colony-wide) brood chambers, called 'gonozooids'. This is in marked contrast to the vast majority of living bryozoans, the Cheilostomata, in which each individual (zooid) forms a brood chamber to house a single embryo.

Cyclostome colonies, like those of all bryozoans, are hermaphroditic. Each individual zooid goes through a male phase, followed by a female phase. Every zooid produces an ovum, but all ova, except those in the gonozooids, are resorbed. The eggs in the gonozooid are presumed to be fertilized by sperm from another colony. Following fertilization, one or two primary embryos within the gonozooid divide numerous times to produce up to 100 secondary embryos (Reed, 1991). Secondary embryos may in turn divide (in *Crisia*) to produce hundreds of tertiary embryos (Robertson, 1903).

Although Borg (1926) found no evidence of secondary fission in the members of the Crisiidae that he studied, this process has been observed in the families Tubuliporidae, Lichenoporidae and Horneridae (Harmer, 1893, 1896, 1898; Borg, 1926). Polyembryony appears to be subsidized by nutrients transferred through a tissue bridge formed by a regressed zooid, which Borg (1926) termed the 'nutritive cylinder'. The embryos in the brood chamber develop into lecithotrophic pelagic larvae, which swim in the water column for a short period (generally < 1 day) upon release. Once the larvae have located appropriate substrata (rocks, algae, etc.), they cement themselves to the bottom and metamorphose into an 'ancestrula', the founding zooid of a new colony. Growth proceeds by asexual budding to produce a modular colony with many repeated units (zooids).

Echinoderms

Larval cloning has been documented in seastars (Bosch *et al.*, 1989; Jaekle, 1994). Descriptions to date have been carried out with material gathered from plankton tows, and the identity of the species to which these larvae belong has not yet been determined. The distinctive morphology of some of the larvae, however, allows them to be assigned to the paxilloid genus *Luidia* with some confidence. Other cloning larvae possess brachiolar arms, indicating that they belong to a non-paxilloid order. This suggests at least two separate origins of larval cloning within the class. Three distinct modes of larval cloning have been documented in asteroid larvae (Bosch *et al.*, 1989; Jaekle, 1994). In paratomous budding, larvae are budded off the tips of the posterolateral arms.

These secondary individuals are generally released as late gastrulae or very early larvae, but occasionally as fully formed larvae. This mode has been observed in larvae presumed to be paxillosid and non-paxillosid. A second mode involves autotomization of the anterior portion of the preoral lobe (essentially reproduction by decapitation). Within a few days, the autotomized preoral lobe generates a coelom using coelomic material from the primary larva and a completely new gut from ectodermal cells. The third mode of cloning consists of budding individuals that are organizationally similar to early embryos from the arm tips. These secondary individuals gastrulate to form a gut and begin to feed within a day or two. To date, the second and third modes have only been observed in presumed paxillosid larvae.

A less certain case of larval cloning in echinoderms has been described in an unidentified brittle star larva, '*Ophiopluteus opulentus*' (Mortensen, 1921, pp. 147–149). In this case, the larva apparently releases a juvenile to the benthos, then returns to the water column where it regenerates a ciliated band and posterior gut and resumes feeding. This post-release larva is presumed to be capable of producing another adult rudiment, although this has not been observed. Again, the larva in question was collected in a plankton tow, and not knowing the species has impeded further observations and experimental studies.

Larval cloning in echinoderms may increase the size of the genet, but probably comes at a cost (Jaekle, 1994). In each of the cloning modes known for seastar larvae, feeding rates of the primary larvae are probably reduced. Careful observation of particle capture in other echinoderm larvae has demonstrated the importance of various regions of the ciliated band in feeding (Hart and Strathmann, 1995). It seems likely that this process is compromised by both paratomous arm budding and loss of the preoral lobe (first and second modes); production of tiny embryo-like buds (third mode) may interfere less with feeding. The apparent mode of cloning by jettisoning a juvenile, noted in the brittlestar example, may also represent a less costly way of cloning as a larva. In this case, pre-metamorphic development of the primary individual is not compromised at all, and larval tissues that would otherwise be autolysed are simply used over again.

Armadillos

Long-nosed armadillos of the genus *Dasypus* are the only known vertebrates to use obligatory polyembryony. We argue (see above) that this is the only way in which they can increase their clutch size because they are limited to a single egg implantation site. Presumably, the ecological conditions for the common ancestor of the *Dasypus* clade favoured the evolution of a single implantation site, which would have two adaptive benefits: (1) it would position the blastocyst in the area most conducive to successful implantation, and (2) it would increase the reliability of implantation in a species with a long pre-implantation period (approximately 4 months: Nowak, 1991).

Nine-banded armadillos (*D. novemcinctus mexicanus*) primarily give birth to identical quadruplets, while twelve-banded armadillos (*D. hybridus*) give birth to octuplets (McLaren, 1982). These numbers are not invariant, however, as others (McLaren, 1982; Hughes, 1989) had earlier claimed. Reduction of viable embryo number sometimes occurs as a result of intra-uterine mortality. Occasionally, *D. n. mexicanus* produces two, three, five or six offspring (Newman, 1913; Buchanan, 1957; Storrs, 1967; Galbreath, 1985), and although *D. hybridus* usually produces 8 or 9, it is capable of producing as many as 12 (von Jhering, 1885, 1886; Fernandez, 1909). Like *D. novemcinctus*, *D. sabanicola* generally produces four offspring, whereas *D. kappleri* produces only two (Galbreath, 1985). Clutch size and polyembryony in the two remaining species of long-nosed armadillos (*D. septemcinctus* and *D. pilosus*) has yet to be examined.

Appendix 2: Cases excluded from our definition

We excluded from Table 1 so-called progenetic aphids (Kennedy and Stroyan, 1959, p. 140), in which several parthenogenetic generations can be telescoped within a mother's body. They are reminiscent of the nested multiplication of the monogenean *Gyrodactylus* (see Appendix 1) with the important distinction that in *Gyrodactylus*, 'Russian dolls' are produced by unequal cleavages of a zygote, whereas each aphid-instar produces an unfertilized egg by meiosis. Hence the 'Russian dolls' in aphids are all genetically distinct from one another, whereas they are genetically identical in *Gyrodactylus*.

We also do not consider cloning at the juvenile stage to be polyembryony, because juveniles usually live under the same ecological conditions as adults (for which their genotype is then proven), as for example in fragmentation in some annelids (Berrill, 1952) or fission in turbellarians (e.g. *Polycelis felina*: de Beauchamp, 1961, p. 95; *Dendrocoelum lacteum*: Koscielski, 1973).

A special type of larval budding occurs in groups in which a free-living larva gives rise to a colonial adult prior to settlement. For example, in *Plumatella* and other freshwater bryozoa (Brien, 1960, p. 1106), pyrosomid tunicates (Fioroni, 1987, fig. 25) and loxosomatid entoprocts (C. Nielsen, pers. comm.), the larva buds several zooids, which remain connected. We therefore interpret these cases to simply represent accelerated colony formation.

Numerous and varied taxa show accidental or 'sporadic' polyembryony (Patterson, 1927). For example, monozygotic twinning occurs at a very low frequency in humans (0.35%: Bulmer, 1970; MacGillivray *et al.*, 1975). In mammals with a clutch size of one, however, 'twinning' is generally associated with increased mortality due to the low and variable birthweights of offspring and the complications of rearing them (e.g. in sheep: McLaren, 1982).

Sporadic polyembryony has been suggested to occur, but to represent aberrant development, in turbellarians (Benazzi and Benazzi Lentati, 1993), gastrotrichs (Hummon and Hummon, 1993) and anthomedusan hydroids (Shostak, 1993). Occasional double-embryos have been observed in scorpions (Berland, 1951, p. 834), grasshoppers (Slifer and Shulow, 1947) and in the oligochaete annelids *Allolobophora* (Dawydoff, 1959, p. 664), *Sparganophilus eiseni* (Hague, 1923), *Tubifex* spp. (Welch, 1921; Penners, 1924) and *Lumbricus* (now *Helodrilus*) *trapezoides* (Kleinenberg, 1879). Although these species frequently show early embryonic bifurcations, these commonly result in malformations (Patterson, 1927). Sporadic twinning therefore appears to be deleterious, although further embryological work is required to clarify this.