

## ASEXUAL REPLICATION AND COLONIALITY IN THE ASCIDIACEA

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The tendency to form colonies of **zooids** is one of the defining characters of the Ascidiacea. Sexual reproduction involves the processes of **meiosis** and **mixis** (fertilization), asexual reproduction involves only **mitosis**, is essentially a growth process, and is better termed **asexual replication**. The confusion with reproduction probably stems from the **ovic** process found in aphids and rotifers, in which unfertilized eggs develop into new individuals without fertilization (i.e. by **apomixis**, *apo* = without). Replication in ascidians is **non-ovic** and involves fully differentiated cells. When the parent replicates using small amounts of tissue, the process is called **budding** (as in ascidian zooids); when the parent divides into halves or large portions it is called **fission** (as in many didemnid colonies). Budding in ascidians generally results in the formation of **colonies**, fission produces **clones**; in colonies the propagules remain in **physiological continuity**, in clones they separate.

Budding processes and resultant coloniality have evolved several times in the Ascidiacea. In CLAVELINIDAE (**Aplousobranchia**) short stolons, arising from the zooid base, swell terminally and develop into buds which grow into new zooids, resulting in zooid clusters. Since the stolons tend to be transitory, the zooid clusters become clones rather than colonies. Stolonial budding is a little more sophisticated in PEROPHORIDAE (**Phlebobranchia**). A succession of buds is produced along the stolons, which survive and provide physiological integration via the colonial vascular system. The buds develop into new zooids, whose degree of aggregation depends on the length of stolons and the distance between consecutive buds. In other aplousobranchs, including DIAZONIDAE (for other reasons is commonly classified in the **Phlebobranchia**) budding involves a regenerative tissue called the **epicardium**, which develops as paired outgrowths from the base of the branchial sac, either side of the pericardium. *Ciona* also has an epicardium, of unknown function, since it does not bud. Aplousobranchs typically have their gut situated in an elongated abdomen or post-abdomen, into which the epicardium extends. Budding, with a strong seasonal component, involves the breakdown and autolysis of the viscera, commencing in the (post-) abdomen. The elongated, degenerated tissue mass then **strobilates** (divides by a series of transverse constrictions), each portion (**strobila**) becoming a bud which, during the growing season or at the onset of the following year's growing season, develops into a new zooid. Because of their zooidal shape and method of budding, the colonies of POLYCLINIDAE tend to form lumps or lobes that are relatively tall. In some HOLOZOIDAE (e.g. *Distaplia*), perhaps pointing to the way in which didemnid budding evolved, only one pair of buds develops, close to the branchial sac.

In DIDEMNIDAE typically a pair of buds, each including epicardial tissue, arise in the oesophageal region of the zooid (there is no post-abdomen). The bud closest to the thorax develops into a new abdomen for it, and that closest to the abdomen develops into a new thorax. The completed zooids separate, resulting in two zooids derived from one parent. This is the 'classical' description of didemnid budding but variations of detail do occur. One advantage of this process is that feeding zooids do not have to degenerate, so that budding is energetically efficient and quick, favouring rapid growth; and a consequence is that the colony spreads laterally, producing a thin layer (so different from a polyclinid colony). An independently evolved, but resultantly analogous method of budding has evolved in the **Stolidobranchia**, that also leads to the formation of flat colonies. There is no epicardium in this order and **palleal** budding takes place from paired origins in the mantle wall. In POLYZOINAE buds grow into new zooids united basally by a thin layer of test, but in BOTRYLLINAE, with immersed zooids (whereon buds tend to be produced in cohorts followed by breakdown on the parent), each generation of zooids becomes arranged in **systems** (as in DIDEMNIDAE and POLYCLINIDAE). To speed up the process in botryllines, well developed buds have already commenced formation of the paired buds

of the following generation.

Colonies and their formation received intensive study during the 1970s and 80s, consequent on three convergent interests: (1) the principles underlying **coloniality**, explored by Beklemishev, became known in the West when his book was translated; (2) the concept of **modularity** developed by Harper's school of plant ecologists was extended to animals; (3) the relative roles of sexual reproduction and asexual replication were examined in the light of 'the costs of meiosis' (i.e. the need for two parents and the dilution of a highly fit genotype) by G.C. Williams and J. Maynard Smith. Beklemishev traced the evolution of colonies from clusters of loosely connected zooids to highly integrated colonies. He recognized that development of the colonial habit was characterized by: (1) diminishing individuality of the zooids; (2) increasing individuality of the colony; (3) the development of colonies within colonies (e.g. the **systems** of ascidians). In ascidians the succession is illustrated, for example, by: **unitary** species > CLAVELINIDAE (bud from stolons but tend to break up into clones) > PEROPHORIDAE (bud from stolons, and retain physiological connection by colonial vascular system) > HOLOZOIDAE and POLYZOINAE (bud contiguous zooids which retain separate exhalant openings to the exterior) > POLYCLINIDAE, DIDEMNIDAE and BOTRYLLINAE (with zooids arranged in systems around a common cloaca). The role of sexual reproduction is to produce a range of genotypes, of which only the best adapted to a given environment will survive. Asexual replication, whether leading to clones or colonies, then rapidly propagates successful genotypes unchanged (although the genetic uniformity of a colony may be modified by somatic mutation or through fusion with a different genotype, which leads to a chimaera).

The evolution of coloniality in ascidians has been driven, to a large extent, by geometry (the characteristics of shapes). As animals grow (as a simple approximation) their ability to capture and absorb food for maintenance, growth and reproduction depends on surface area and therefore increases as the square of their linear dimensions; but the energetic requirements of these processes is related to body mass and increases as the cube of linear dimensions. Therefore, the smaller the animal the energetically more efficient it is. To become larger, the options are to increase the efficiency of the food capturing apparatus (the branchial sac) or to develop colonies, in which food capture is by small zooids and colony size tends to increase in two rather than three dimensions. As the colonies become better integrated, so zooids tend to become smaller (cf. CLAVELINIDAE and POLYCLINIDAE, or POLYZOINAE and BOTRYLLINAE).

In an analogous manner, the evolution of colony form has been largely driven by hydrodynamic forces. Ascidian zooids expend energy when feeding by drawing water through their oral opening, filtering it through their branchial sac, and expelling it through their atrial opening. It is vital not to re-inhale previously filtered water, which will be both devoid of food particles and contaminated by faecal and/or excretory products (known to be harmful). This has led to the formation of systems, in which energetically efficient zooids with small inhalant openings exhale into a **common cloacal cavity** and discharge a relatively powerful flow through a large **common cloacal opening**. This outflow will carry above the boundary layer and the water will not be re-inhaled. The arrangement then benefits from the hydrodynamic properties of the boundary layer: overflowing water (since ascidians tend to flourish in regions of good water flow) will tend to draw water out of a large opening—but not a small one—by the phenomenon of **viscous entrainment**. Moreover, if the common cloacal aperture is elevated, as in many didemnids, it will project into faster flowing water away from the immediate boundary. Since the faster water flows, the lower its static pressure, there will be both enhanced inflow through the oral openings at the surface and enhanced outflow from the elevated common cloacal aperture. This is known as **Bernoulli's Principle** (the same one as explains how lift is generated as airspeed accelerates over the upper surface of an aeroplane's wings). What might perhaps be regarded as the ultimate system, in which the colonies have the form of squat chimneys with one wide, terminal opening, has evolved twice—independently in both DIDEMNIDAE (*Didemnum molle*) and BOTRYLLINAE (*Botrylloides magnicoeca*). These evolutionary trends are paralleled in

other colonial or modular taxa, viz. bryozoans and sponges.

The methods of budding and colony expansion in DIDEMNIDAE seem to have evolved to facilitate a rapid growth rate, and produce a colony that can spread and overgrow competitors. A colony of roughly circular form will, however, suffer an increasing constraint to its growth rate. This is because, while zooid numbers depend on colony area—proportional to (radius)<sup>2</sup>—expansion (dependent on actively budding zooids near the margin though supported by metabolites transmitted from interiorly situated feeding zooids) is consequent on circumference—simply proportional to radius. Thus the larger the colony, the smaller its circumference to area ratio, and the rate of formation of new zooids inevitably decreases. The solution is to regenerate small colony size by fission (cloning) as occurs spectacularly in the tropical *Diplosoma virens* and many other didemnids. The processes of asexual replication have then gone full cycle, from budding to form colonies to fission to create clones. It can also be shown algebraically that the chances of survival of the genotype (**genet**) will improve as the number of dispersed **ramets** (sibling colonies or clonemates) increases [if an annual mortality coefficient (e.g. 0.03) of single colonies is  $M$ , for a pair of ramets it is  $M^2$  (hence 0.009) and for  $n$  ramets it is  $M^n$  though, of course, the mortality rate itself declines with increasing size].

### Selected Bibliography

Budding methods were described many decades ago (especially Berrill, 1935), though Bell (1982) and Hughes (1989) also summarise budding/cloning methods, while there are significant papers by Kott (1982) and Nakauchi (1982).. The interest in cloning and coloniality (in Europe and the US) developed especially in the 1970s (stimulated by the English translation of Beklemishev, 1969, and Harper's ground-breaking plant ecology book, 1977) and '80s (as the references show), with some superb books (Williams 1975 (the early chapters are relevant), Bell 1982, Hughes 1989), conference volumes (Larwood & Rosen 1979, Jackson *et al.* 1985, Harper *et al.* 1986) and papers (Chapman & Stebbing 1980, Hughes & Cancino 1985, Ryland *et al.* 1985, Mackie 1986, Pearse *et al.* 1989, Stoner 1989). There are also papers by these and other authors, not itemised, in the edited volumes listed below.

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