A Functional Biology of Clonal Animals

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Functional Biology Series: Foreword

General Editor: Peter Calow, Department of Zoology,
University of Sheffield, England

The main aim of this series will be to illustrate and to explain the way organisms 'make a living' in nature. At the heart of this — their *functional biology* — is the way organisms acquire and then make use of resources in metabolism, movement, growth, reproduction, and so on. These processes will form the fundamental framework of all the books in the series. Each book will concentrate on a particular taxon (species, family, class or even phylum) and will bring together information on the form, physiology, ecology and evolutionary biology of the group. The aim will be not only to describe *how* organisms work, but also to consider *why* they have come to work in that way. By concentrating on taxa which are well known, it is hoped that the series will not only illustrate the success of selection, but also show the constraints imposed upon it by the physiological, morphological and developmental limitations of the groups.

Another important feature of the series will be its *organismic orientation*. Each book will emphasize the importance of functional *integration* in the day-to-day lives and the evolution of organisms. This is crucial since, though it may be true that organisms can be considered as collections of gene-determined traits, they nevertheless interact with their environment as integrated wholes and it is in this context that individual traits have been subjected to natural selection and have evolved.

The key features of the series are, therefore:

1. Its emphasis on whole organisms as integrated, resource-using systems.
2. Its interest in the way selection and constraints have moulded the evolution of adaptations in particular taxonomic groups.
3. Its bringing together of physiological, morphological, ecological and evolutionary information.

P. Calow

Preface

It is natural to think of life and sex as inseparable, yet most major groups of living organisms include species that reproduce in ways which, contrary to normal sexual reproduction, perpetuate the maternal genome. In other words, they clone. Familiar examples are bacterial plaques growing on agar plates, a hillside covered by the vegetative spread of bracken, or a plant smothered in aphids. If cloning were simply an alternative method of reproduction, there would be little more to discuss. The genetic identity among clonal progeny, however, bestows special ecological and genetical advantages, while at the same time inflicting certain evolutionary disadvantages if sex is lost from the life cycle.

In those species that retain normal sex, cloning may be regarded as extended somatic growth, so providing an interesting perspective on their life history. The clonal 'soma' can be in several places at once and each member of the clone has the potential to replicate itself. The total risk of annihilation by point-sources of mortality is reduced, the colonizing potential is increased and the clonal 'soma' is free to grow beyond the bounds set by physical constraints acting upon individuals.

These principles apply as much to rotifers, for example, as they do to aphids, corals, fish or lizards. For evolutionary biologists, clonal organisms provide valuable comparative material in their quest to understand the significance of sexual reproduction, while for experimental biologists they provide a means of controlling genetic variation among their subjects.

The aim of this book is to draw together current ideas of clonal biology, applicable to all major types of clonal Metazoa. Chapter 1 explains the nature of cloning in broad terms and summarizes its occurrence among the Metazoa. Chapter 2 examines the diverse mechanisms and Chapter 3 the consequences of cloning. Chapter 4 characterizes the life histories of clonal

animals with particular reference to the presence of sex, its evolutionary significance and its timing in the life cycle. Chapter 5 reviews the population genetics of clonal animals whose life cycles incorporate normal sex. Chapter 6 considers the ecological and evolutionary characteristics of clonal animals lacking normal sexual capabilities, including hybridogenetic and gynogenetic species that bridge clonal and aclonal life. Chapter 7 examines the life history and population biology of modular animals, which in some ways resemble plants more closely than they do other animals. I hope, by summarizing the scattered information on this diverse subject, to stimulate further interest in the evolutionary significance, ecological importance and experimental usefulness of clonal metazoans.

Contents

1 *The nature of clones*

1.1 INTRODUCTION

The term *clone* is derived from a Greek word *klon* meaning twig or slip and may have originated from the idea of propagating desirable strains of plants by taking cuttings. Common usage of the term is usually in the context of artificial propagation, not only by cuttings but by an impressive variety of techniques enabling exact genetic replicas of the original to be made over and over again. Often these techniques involve quite unnatural processes such as tissue culture; in this way the cervical carcinoma cells of Henrietta Lacks (HeLa), who died in 1951, are propagated in laboratories throughout the world (Jones *et al.*, 1971), while horticulturally valuable plants are grown from meristem cultures. These techniques propagate the whole genome, but with genetic engineering and the synthesis of monoclonal antibodies, methods are now available for cloning subcomponents of the genome and their products down to the level of single genes.

Other techniques involve natural processes; special strains of brewer's yeast (Figure 1.1) are cloned by providing the right conditions for continued mitotic cell division, while strawberry plants are cloned from their runners. In fact natural cloning methods are even more diverse than those devised by man. Clonal processes occur naturally and commonly among micro-organisms, higher plants and animals, but it is only among micro-organisms and higher plants that they have been exploited commercially. This is because farmed animals lack a natural ability to form clones and artificial methods have not yet been devised for them. Great prizes would be won by overcoming these problems, indeed considerable efforts were once made to breed a clonal variety of turkey from the Beltsville strain, which produces a

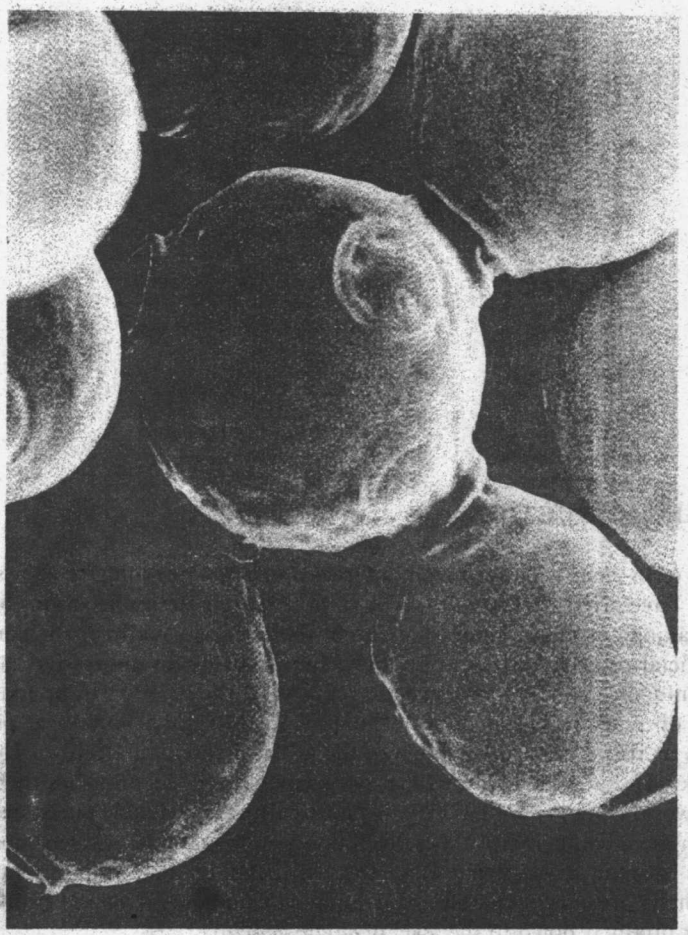

Figure 1.1 Brewer's yeast, a commercially important clone. Cells are propagated by mitotic division. (Electron micrograph supplied by A.H. Rose.)

proportion of eggs that hatch without fertilization (Poole and Olsen, 1957). Unfortunately, the sex-chromosome mechanism of birds ensures that all unfertilized eggs develop into males, so the breeding programme was severely limited. Natural cloning in animals, however, has been put to good use for scientific purposes. Many clonal invertebrates are small, reproduce rapidly and are easily cultured, making them ideal subjects for the experimental investigation of general biological problems.

1.2 THE CLONING PROCESS

A clone is an assemblage of individuals that are genetically identical by descent (Bell, 1982). This definition is open to broad interpretation, depending on what are included as individuals; not only the whole body, but also cells, organelles, or even components of the genome could be regarded as individuals that replicate and preserve their genetic identity. Every metazoan undergoes a phase of development and growth by mitotic cell division. Apart from rare mutations, therefore, all the somatic cells are genetically identical and in this sense the body could be regarded as a clone. There is, however, a fundamental difference between a clone of HeLa carcinoma cells and the assemblage of cells that once comprised the body of Henrietta Lacks: the cultured HeLa cells function independently of one another, whereas the cells of her body functioned in an organically integrated manner. As will be discussed later, the degree of functional independence among the replicated individuals is an important consideration when defining a clonal process. It is appropriate to note that some cell biologists use identity of genetic function rather than genetic composition as the criterion for cloning. When an embryo develops, the genome of different cells becomes differentiated by the activation of tissue-specific loci, each functional type giving rise to a 'clone' of cells characteristic of a certain tissue (Mintz, 1971).

All eukaryotic cells contain self-replicating organelles, the mitochondria, which possess their own DNA. As they multiply within the cell, the mitochondria preserve their genetic identity and, although dependent on the intracellular environment and the controlling influence of the nucleus, they are the functionally independent products of a sub-cellular cloning process. Mitochondria themselves are probably the descendants of bacteria that symbiotically invaded eukaryotic cells and continued a bacterial type of clonal replication (Margulis, 1981). The clonal nature of mitochondria has provided evolutionary biologists with an important tool. Since mitochondria are passed from a mother to her eggs independently of the nucleus, they escape genetic recombination and their clonal propagation continues uninterrupted in the maternal lineage. Rare mutations slowly accumulate in the mitochondrial DNA and the resulting differences provide a measure of evolutionary divergence among lineages. If the mutation rate is known, the evolutionary time scale can be estimated.

Even at the level of the genome, two modes of clonal replication may operate (Buss, 1985). One is the familiar self-replication of strands of DNA producing identical copies of the genome. The other is the more enigmatic, autonomous replication of small, mobile subunits of the genome. These transposons can break away and reincorporate themselves into different chromosomal locations, carrying genetic material with them. Indeed it may

prove to be the case that transposons play an important part in the evolution of genomes (Doolittle and Sapienza, 1980; Campbell, 1982).

This book however, is concerned with individuals at the organismal level, where a clone is an assemblage of genetically identical animals, for example the aphids infesting a host plant, or the anemones covering an intertidal rock face. A modular colony, such as a tuft of hydroid or a coral head, is not regarded as a clone, because although the modules (polyps and zooids) are self-replicating, genetically identical units, they are organically interdependent (section 2.2). This, of course, is only a matter of degree; some cnidarians produce separate polyps that live a completely independent existence, while in others the separated polyps remain aggregated and are functionally co-operative. Because of this gradation, certain authors have regarded modular colonies as clones (Hughes and Cancino, 1985). All such colonies however, are capable of regeneration from fragmented parts and in fact this is a common natural process. Here there are two levels of replication, modular and colonial. Confusion is avoided by regarding the colony as a modular individual, which by fragmentation may form more colonies that together constitute a clone. The definition given earlier therefore needs amending to include this qualification: *a clone is an assemblage of genetically identical individuals that can function and survive on their own* (Jackson *et al.*, 1985). The term 'cloning' means the process of multiplication, resulting in the production of genetically identical offspring. 'Clonal growth', depending on the context, can mean the population growth resulting from cloning, or the increase in biomass resulting both from clonal reproduction and individual growth. In the second sense, individuals are regarded as fragments of the clonal 'soma'. Sexual reproduction is taken to mean mictic reproduction, in contrast to the amictic process of cloning.

Finally, the word population requires scrutiny. Clones or individuals may be the units of population, and in the case of modular colonies the modules themselves may represent a third unit. Distinction between these viewpoints could be important. For example, a geneticist may be interested in the number of genomes (clones) comprising a population, whereas an ecologist may be more interested in the number of individuals which, perhaps, are competing for some resource. Often, the geneticist and ecologist would be interested in both the number of clones and of individuals. Definitions and synonyms are summarized in Table 1.1

1.3 TWO TYPES OF CLONING

Animals clone in two fundamentally different ways: by agametic reproduction, popularly termed asexual reproduction, and by parthenogenesis, including hybridogenesis and gynogenesis. Agametic reproduction involves

Table 1.1 Terms and synonyms

Term	*Definition*	*Synonyms/Comments*
Clone	An assemblage of individuals, genetically identical by descent, organically separated and able to function independently	Genet, genetic individual
Individual	A member of a clone, may be unitary or modular	Somatic individual, ramet, module (in this book module is reserved for colonial animals)
Module	The unit of colonial architecture in aquatic invertebrates; product of growth, budding or partial fission (e.g. aquiferous modules of sponges, polyps of cnidarians, zooids of bryozoans)	
Colony	An assemblage of organically and functionally integrated modules	Modular colony, modular individual, ramet, regarded as a clone of modules by some authors
Unitary	Non-modular, applies to all non-colonial individuals	Harper (1977) reserved this term for aclonal organisms
Clonal growth	The numerical result of clonal reproduction, expressed either as number of individuals, or collective biomass	
Clonal soma	The collective biomass of a clone	
Clonal reproduction	The creation of new individuals within a clone, occurs by agametic reproduction or parthenogenesis	Clonal growth, cloning
Agametic reproduction	The creation of new individuals by any process of somatic division (fragmentation, fission, budding, polyembryony, somatic embryogenesis)	Asexual reproduction

Table 1.1 continued

Term	*Definition*	*Synonyms/Comments*
Gametic reproduction	The creation of new individuals from eggs (parthenogenesis and bisexual reproduction)	
Bisexual reproduction	Reproduction from fertilized eggs	Normal sexual reproduction, basic bisexual reproduction, sexual reproduction, mictic reproduction
Parthenogenesis	Reproduction from unfertilized eggs	Included with asexual reproduction by some authors
Modular iteration	Multiplication of modules within a colony by budding or partial fission	Colonial growth

fragmentation, fission and budding, whereas parthenogenesis involves the development of unfertilized eggs. In the former, each offspring develops from a group of cells, but in the latter from one cell, a difference of fundamental biological importance (section 3.2.1).

Cloning occurs in two thirds of the metazoan phyla (Bell, 1982; Hughes and Cancino, 1985). Agametic reproduction and parthenogenesis are usually mutually exclusive (Table 1.2) for an obvious reason. Agametic reproduction is feasible only in those animals with a relatively simple morphology that can be regenerated quickly from incomplete or rudimentary parts and it is hampered by hard, complicated skeletal structures. Soft-bodied invertebrates and colonial forms made up of repeated sub-units (Chapter 7) are therefore the ones which clone by agametic reproduction. The others, including the arthropods with their intricate exoskeleton and the vertebrates with their great morphological complexity, clone by parthenogenesis or, exceptionally, by polyembryony (Chapter 2).

1.4 RECOGNITION OF CLONES

1.4.1 Detection of cloning

Unfortunately for the biologist, clonal animals seldom appear to be morphologically different from their aclonal relatives. This is hardly surprising: the only fundamental difference between clonal and aclonal lineages

Table 1.2 Incidence of agametic cloning, parthenogenesis and bisexual reproduction among metazoan phyla

Phylum	*Agametic cloning*	*Parthenogenesis*	*Bisexual reproduction*
Porifera	+	0	+
Mesozoa	+	0	+
Coelenterata	+	0	+*
Ctenophora	+	0	+
Platyhelminthes	+	(rare)	+†
Nemertea	+	(rare)	+†
Gastrotricha	0	+	+‡
Rotifera	0	+	+†
Kinorhyncha	0	0	+
Nematoda	0	+	+†
Nematomorpha	0	0	+
Acanthocephala	0	0	+
Gnathostomulida	0	0	+
Annelida	+	(rare)	+
Mollusca	0	+	+†
Arthropoda	0	+	+†
Onychophora	0	(rare)	+†
Pogonophora	0	0	+
Sipuncula	+	0	+
Echiura	0	0	+
Priapulida	0	0	+
Tardigrada	0	+	+†
Pentastomida	0	0	+
Phoronida	+	0	+
Bryozoa	+	0	+
Entoprocta	+	0	+
Brachiopoda	0	0	+
Echinodermata	+	(rare)	+
Chaetognatha	0	0	+
Hemichordata	+	0	+
Chordata	0	+	+†

+, present; 0, absent.
* rarely 0.
† sometimes 0 if clonal.
‡ 0 if clonal.

is that parents and progeny in the former are genetically identical whereas in the latter they are not. Once animals are known to be clonal, it may be possible in some cases to recognize them by their individual characteristics, for example certain clonal lizards can be recognized by their dorsal colour patterns (Zweifel, 1965), but there are no external characteristics that are specifically correlated with cloning. When animals reproduce by fragmentation, fission or budding, they are certain to be clonal because

these processes are the result of mitotic cell-division. When animals reproduce by parthenogenesis, however, they are not necessarily strictly clonal. This is because parthenogenesis can involve any one of several different cytogenetic mechanisms during oogenesis, not all of which preserve the maternal genome (White, 1973). Those that involve some genetic recombination, however, always increase the proportion of homozygosity in each generation, so that if reiterated they will eventually produce completely homozygous clones (Chapter 2).

Even parthenogenesis itself is not easy to determine. Usually it is inferred from the apparent absence of males. However, males may be rare, inconspicuous, short lived or more difficult to collect than the females and the only way to be sure of parthenogenesis is to breed the females in captivity (Figure 1.2). This must be done for more than one generation since many animals can store sperm for a long time and they may have mated before capture. If breeding in captivity is not possible, then parthenogenesis can only be confirmed by cytological studies of oogenesis. A partial test, however, may be made by using the electrophoresis of allozymes to examine populations for genotypic uniformity and departures from Hardy–Weinberg equilibrium and to examine laboratory cultures for identity among parents and offspring at variable loci.

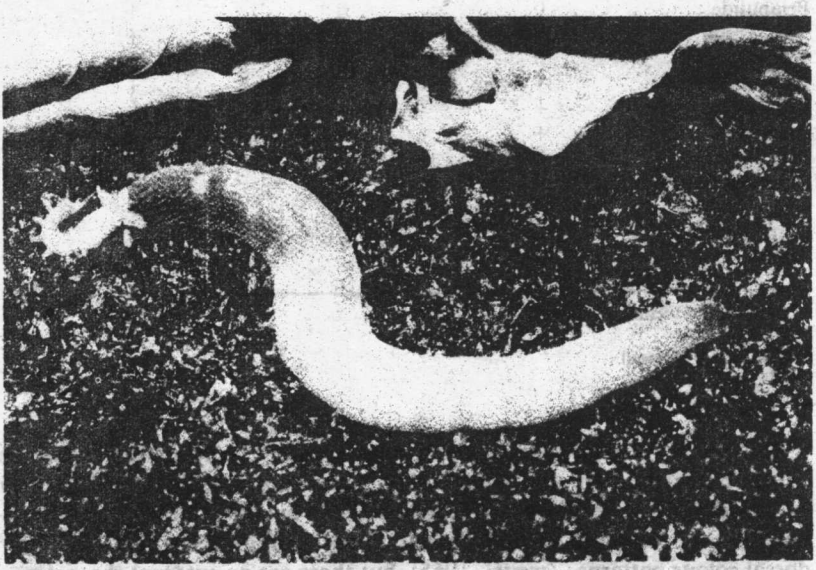

Figure 1.2 A recently discovered clonal animal *Epiperipatus imthurni*. Several parthenogenetic generations have been raised in the laboratory. No males occur. The mother is giving birth to her clonal offspring (left). (From Read, 1985.)