# Genotypic Variation of Polypide Regression, Growth Rate and Colony Form in the Marine Bryozoan *Electra pilosa* (L.)

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For my parents

## Declaration

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## Abstract

Polypide regression has to date been a markedly neglected aspect of bryozoan biology. The term refers to the cycle of continual degeneration and regeneration of polypides within their zooids which has been reported for several bryozoan species. In the present study, both the effects of genotype and of different food concentrations on the life spans of polypides were investigated in the marine bryozoan *Electra pilosa* (L.) under controlled laboratory conditions. The methodology employed allowed replication of genotypes and their simultaneous subjection to different experimental treatments, thus yielding valuable information on the extent to which the particular traits are genetically controlled. Polypide life spans were significantly different between genotypes, and decreased significantly across all genotypes with increasing food concentration. This supported the expectation that a richer food supply should accelerate the ageing process in the stomach epithelium cells. It is suggested that polypide longevity - in the absence of other stimuli - is controlled by food supply, acting on a largely genetically determined background.

The present study also shows that both whole-colony growth rate and colony form (shape) vary significantly between genotypes, and are highly heritable. Final experimental colony size of colonies grown from equal sizes in the same tank differed almost sixfold between genotypes, after a growth period of only 43 days. Similarly, colony form showed strong continuous variation

between genotypes, ranging from stellate shapes to lobate, almost subcircular colonies. The variation observed probably accounts for much of the phenotypic plasticity of colony form described in the literature. Its potential implications for spatial competition in marine fouling communities are discussed.

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# CHAPTER 1: GENERAL INTRODUCTION

## 1.1 The Bryozoa: General biology, taxonomy and systematics

### 1.1.1 TAXONOMY AND FOSSIL RECORD

In the early days of taxonomy, the Bryozoa were regarded by naturalists as part of the plant kingdom, and, along with the Coelenterata, classified as "zoophytes". The first illustration of a bryozoan dates back as far as 1558 (by Rondelet, cited by Hyman, 1959), but the animal status of the Bryozoa should remain disputed for another 200 years and more. Even the great taxonomists of the 18th century such as Linnaeus and Cuvier continued to use the term zoophyte, despite the fact that the animal status of the taxon had become largely accepted in their times.

In the 1830s, Thompson and Ehrenberg, independently of each other and within only one year's time, created the terms "Polyzoa" and "Bryozoa" respectively, both referring to one and the same taxonomic group. By the middle of the 19th century, it was widely accepted that the phylum, as then defined, comprised two basic types of morphologies: in some animals, the anus opened within the circlet of tentacles, in some outside. On this basis, Nitsche introduced in 1869 the distinction of Bryozoa Ectoprocta and Bryozoa Entoprocta; the latter were given the status of a separate phylum in 1888.

Presently, these terms have more or less been superseded and replaced by the phyla Bryozoa and Kamptozoa respectively.

With about 5000 extant species described, and probably in excess of another 5000 remaining to be described (Horowitz & Pachut, 1992), the Bryozoa constitute by no means a numerically minor group. The number of cryptic species is difficult to estimate, but recent research suggests that it might still be rather high (Jackson & Cheetham, 1992).

Due to the existence of calcified exoskeletons, the fossil record of the Bryozoa is remarkably good; early specimens date back as far as the Lower Ordovician (approx. 490 million years), and the number of fossil species described amounts to around 16000 (Remane *et al.*, 1986). Fossil bryozoans are of great value for the characterisation of geological formations, and the economic implications of this (e.g. in oil prospecting) are only just beginning to be realised.

Probably the best description of a bryozoan is that of a "modular machine" (McKinney & Jackson, 1992); again, all but a few exceptions form colonies, consisting of modules called zooids. At its simplest, a zooid can be described as a functional unit consisting of the body wall which is termed "cystid", and which may or may not be calcified, and a feeding structure consisting of a food capturing apparatus and an alimentary tract, together termed "polypide" (Fig. 1.1 A). The food capture apparatus is referred to as the lophophore, and constitutes one of the main taxonomic criteria for the relationship with the Phoronida and the Brachiopoda; together, the three lophophorate phyla are referred to as Tentaculata. Remane et al. (1986) class the Tentaculata as protostomians and as

Archicoelomata (together with the Hemichordata and the Echinodermata) in the classification according to the coelomic structure.

The Bryozoa comprise three Classes: the Phylactolaemata (a small group of freshwater species), the mostly fossil Stenolaemata, and the ecologically extremely successful Gymnolaemata, which comprise nearly all of the extant species. The taxonomic characteristics of the three classes are compiled in Table 1.1.

### 1.1.2 GENERAL BIOLOGY

Colony growth is achieved by asexual budding of daughter zooids; the resulting growing edge of the colony consists of cuticle, epithelium and a double peritoneum. Apical cells stretch the cuticle by intussusception of new material; the zone of actual colony growth is situated directly behind the zone of cuticular expansion. Calcification, where present, occurs in the central, fibrillar part of the cuticle by deposition of calcium carbonate crystals onto the protein matrix (Ryland, 1970).

All bryozoans feed by producing water-currents with their lophophores; the lophophore consists of tentacles bearing cilia that beat obliquely downwards and outwards, producing a water current aimed at the orifice. Food particles can thus be directed at the orifice and ingested, a method that has been adequately described as "impingement feeding" (Ryland, 1970).

Table 1.1: Taxonomic Characteristics of the Bryozoa (after Ryland, 1970, and McKinney & Jackson, 1992)

**Class Phylactolaemata**
- zooids cylindrical and uncalcified
- lophophore horseshoe-shaped with epistome
- coelom continuous between zooids
- 12 genera described, exclusively freshwater species

**Class Stenolaemata**
- zooids elongate cylindrical, basal and vertical walls calcified, long axis at angle to colony growth direction
- interzooidal communications present
- polypide enclosed by membranous sac, which supports evagination of polypide through orifice
- largely fossil

*Order Trepostomata*
- colonies encrusting or erect
- elongate autozooids with basal diaphragm
- extrazooidal skeletons in some species
- mostly fossil, possibly extant

*Order Cystoporata*
- colonies encrusting or erect
- basal diaphragms may or may not be present
- autozooids with thickened strip ("lunarium") along one side of cystid wall
- fossil

*Order Cryptostomata*
- colonies erect or bilaminate sheets
- no communication pores
- autozooids generally with hemisepta (*i.e.* incomplete lateral partitions)
- fossil

*Order Fenestrata*
- colonies erect
- commonly with hemisepta, no communication pores, heterozooids present
- extensive extrazooidal skeletons
- fossil

*Order Cyclostomata*
- colonies encrusting or erect
- autozooids commonly long, skeletal structure typically laminated
- gonozooids common
- fossil/extant

**Class Gymnolaemata**
- zooids generally box- or sac-shaped, with long axis usually parallel to colony growth direction
- zooidal walls either organic or entirely calcified
- interzooidal communications through funicular network
- vertical or frontal walls deform to evaginate polypide
- fossil and extant

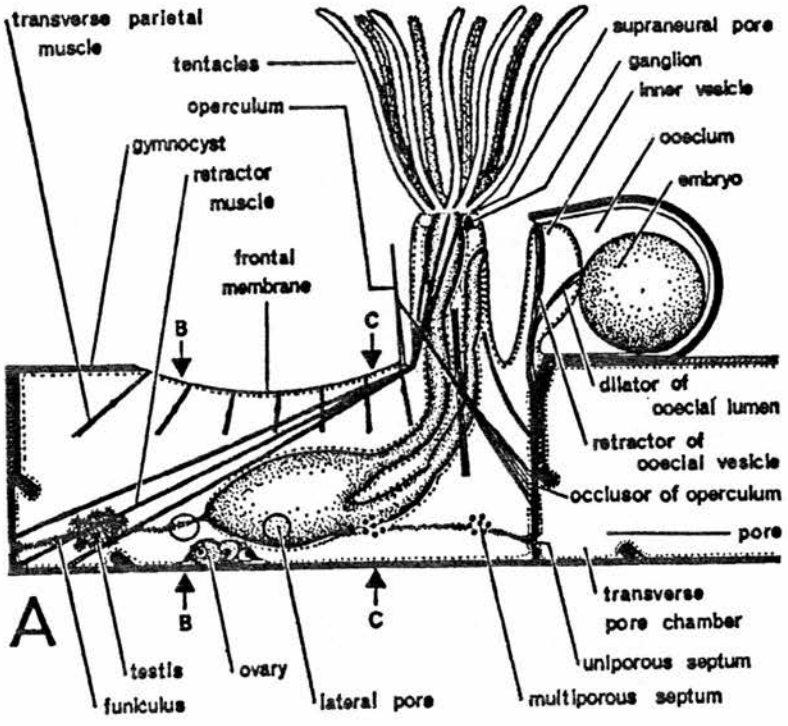
*Order Ctenostomata*
- zooidal walls uncalcified
- orifice terminal, generally pleated collar
- heterozooids absent
- fossil and extant

*Order Cheilostomata*
- zooid walls calcified
- orifice frontal, with operculum
- Suborder Ascophora: frontal walls fully calcified, polypide evaginates by means of compressible sac under the frontal shield
- Suborder Anasca: frontal walls partly or wholly uncalcified, polypide evaginates through increase in coelomic pressure induced by parietal musculature

Integration of individual zooids within the colony occurs at different biological levels: behavioural integration of polypide feeding activity can be achieved by means of a colony-wide nervous system which interconnects zooids (e.g. Thorpe *et al.*, 1975a, b); integration of feeding currents over parts of colonies, or even whole colonies, has also been reported (Lidgard, 1981; Winston, 1978), as has translocation of nutrients to non-feeding parts of the colony (e.g. Best & Thorpe, 1985). In the order Cheilostomata since the late Jurassic, a striking trend towards increased total integration has been noted (Boardman & Cheetham, 1973). This can be measured as the average of six series of morphologic characters (zooid walls, interzooidal connections, extrazooidal parts, astogeny, morphologic differences between polymorphic zooids and positional characteristics of polymorphic zooids).

As a consequence of specialisation of function, zooidal polymorphism has occurred in a variety of species, notably in the Order Cheilostomata. These specialised zooids, also referred to as heterozooids, have lost their ability to feed and taken over functions of mechanical stabilisation, protection from fouling and predation, reproduction or even locomotion. Examples for polymorphic zooids include avicularia, vibracula and spinozooids, all of which serve for mechanical protection of the colony by means of spiny processes, male and female gonozooids (Fig. 1.2 B-E), and kenozooids such as the pore chambers between adjacent zooids (which serve as structural support for the colony) (Fig. 1.1 A). The feeding zooids, which usually make up the majority of a colony's zooids, are termed "autozooids" (e.g. Fig. 1.2 A).

Fig.1.1
A: Zooid structure in a typical anascan cheilostome zooid (from Ryland, 1970).
B: Lateral view of cyphonautes larva (*Electra pilosa*, from Zimmer & Woollacott, 1977a)


transverse parietal muscle
tentacles
operculum
gymnocyst
retractor muscle
frontal membrane
B
C
supraneural pore
ganglion
inner vesicle
oocium
embryo
dilator of oocial lumen
retractor of oocial vesicle
occlusor of operculum
pore
transverse pore chamber
uniporous septum
multiporous septum
lateral pore
ovary
testis
funiculus
A

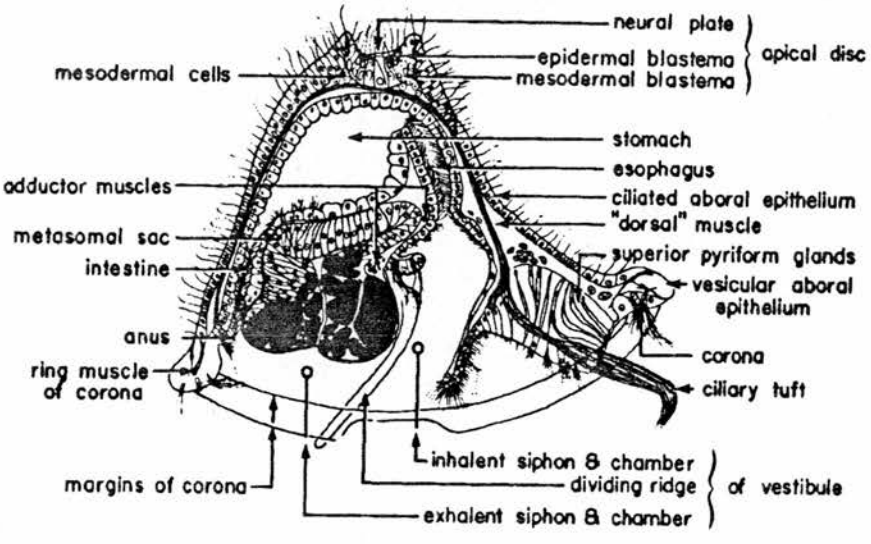
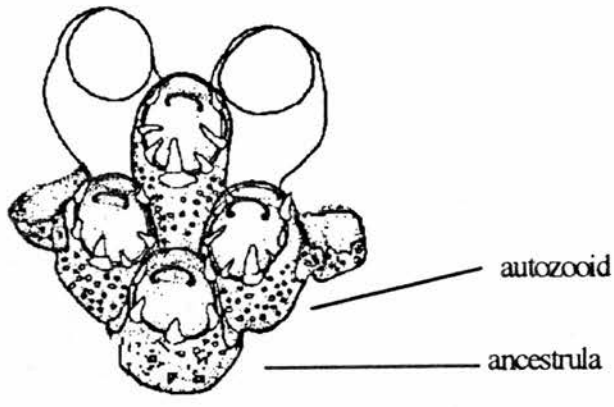
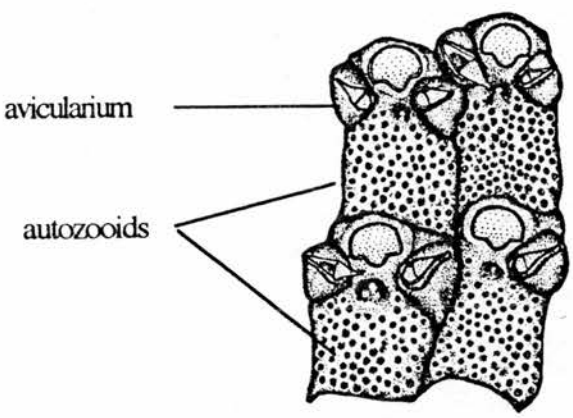

neural plate
epidermal blastema
mesodermal blastema
apical disc
mesodermal cells
stomach
esophagus
ciliated aboral epithelium
"dorsal" muscle
superior pyriform glands
vesicular aboral epithelium
corona
ciliary tuft
adductor muscles
metasomal sac
intestine
anus
ring muscle of corona
margins of corona
inhalent siphon & chamber
dividing ridge
exhalent siphon & chamber
of vestibule
B

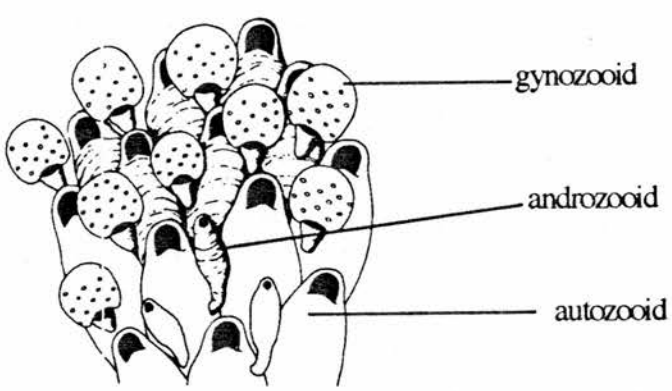
Fig. 1.2 Zooidal polymorphism in bryozoans.
A: Autozooids and ancestrula of *Electra pilosa* (from Ryland & Hayward, 1977)
B: Adventitious avicularia, *Schizoporella unicornis* (from Hayward & Ryland, 1979)
C: Dwarfed gynozooids and androzooids, *Celleporella hyalina* (from Silen, 1977)
D: Vibracula, *Caberea boryi* (from Ryland & Hayward, 1977)
E: Spinozooids, *Membraniporella aragoi* (from Silen, 1977)


autozooid
ancestrula

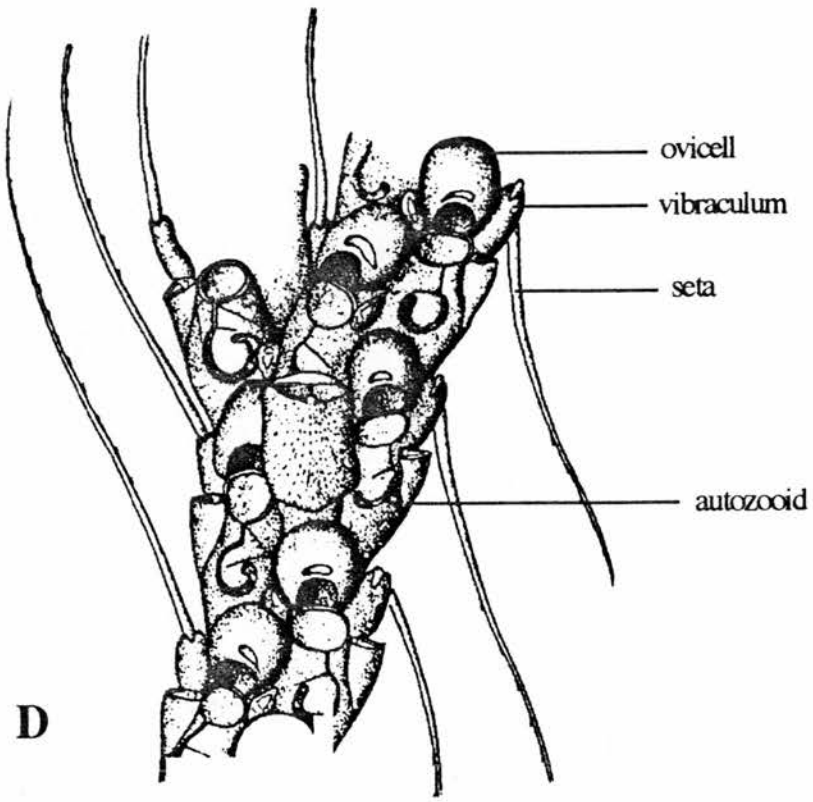
**A**


avicularium
autozooids

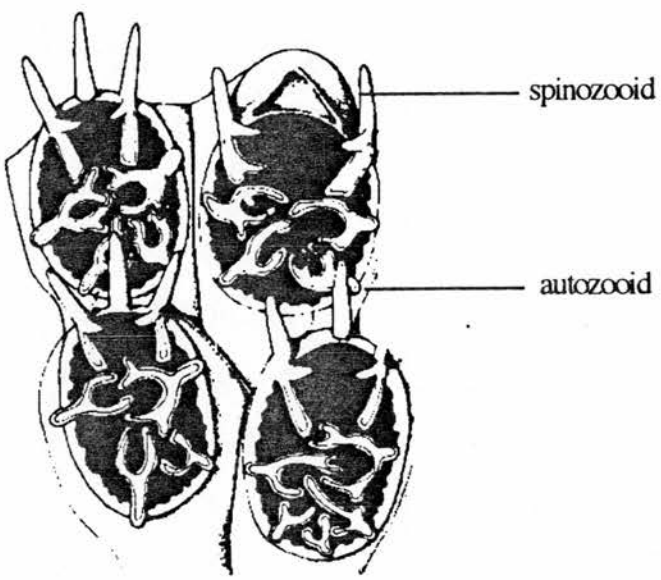
**B**


gynozooid
androzooid
autozooid

**C**




**D**




**E**

### 1.1.3 ECOLOGY

Bryozoans are aquatic organisms and, with a few exceptions (e.g. Bushnell, 1992), they are all sessile. Their habitats include freshwater and brackish environments, but the overwhelming majority of species is marine. Most species are found on the continental shelves, preferably in the intertidal and upper sublittoral, but some have colonised abyssal and even hadal regions (Remane *et al.*, 1986).

### 1.1.4 REPRODUCTION

Bryozoans are generally assumed to be colonial hermaphrodites; dioecy has not been reported for the phylum (Ryland & Bishop, 1993). Colonial hermaphroditism either occurs in the form of zooidal hermaphroditism, with both male and female gonads developing in the same zooid, or as zooidal gonochorism, with testes and ovaries in separate zooids within the same colony (Reed, 1991). Protandrous, protogynous and simultaneous hermaphroditism all have been reported in bryozoans. Gonochoric bryozoan species usually exhibit sexual dimorphism.

Information on reproductive seasonality in bryozoans - although fragmental - suggests that seasonality is largely a function of the geographic and bathymetric distribution of a species. Spatially restricted boreal and temperate zone species typically exhibit markedly seasonal reproduction; cosmopolitan temperate species exhibit greater flexibility, whilst tropical species generally reproduce more or

less continously, albeit at lower levels of reproductive output (Reed, 1991).

Fertilization in the Bryozoa was not understood until comparatively recently, and up to 1966 it was believed that self-fertilization was the rule. Silén (1966) then described the release of sperm through terminal pores in the tentacles of the anascan cheilostome *Electra posidoniae*. In a further study (Silén, 1972) he extended his observations to a number of cyclostome, ctenostome and cheilostome species, all of which were found to release sperm through tentacle pores. It was therefore concluded that outcrossing does occur in bryozoans; this is supported by population biological data, which suggest that allele frequencies in bryozoan populations generally conform to Hardy-Weinberg expectations (Reed, 1991). Selfing, however, is not unusual and has now been described for several gymnolaemate species (Maturo, 1991). Fertilization in non-brooding species, as described by Silén (1966) for two species of *Electra*, occurs presumably either within or directly outwith the intertentacular organ, a coelomoduct ending in a coelomopore between two dorsomedial tentacles. Generally, however, it is believed that external fertilization is the rule in oviparous species, with eggs being fertilized during discharge (Ryland & Bishop, 1993).

The overwhelming majority of species do, however, brood lecithotrophic larvae (Reed, 1991); for these, fertilization has not been described, but it can be assumed to take place during the transfer of the egg to the brood chamber (Ryland, 1976). The most common type of brood chamber is the ovicell or ooecium - a spherical chamber situated at the distal end of

the maternal zooid, and which accommodates the growing embryo. The embryo remains within the brood chamber until the larva is fully developed.

Larval forms comprise three distinct types. First, the shelled, planktotrophic cyphonautes larva of non-brooding species (e.g. *Electra* sp. or *Membranipora* sp., which has a triangular, bilaterally compressed shell, a fully developed digestive tract (Fig. 1.1 B) and is usually pelagic for several weeks (Ryland & Hayward, 1977). Second, the shelled, lecithotrophic larva (such as that of *Flustrellidra hispida* ) the shell of which is of more or less rectangular shape, with a strongly reduced, functionless digestive tract, and a presumably brief pelagic phase. Third, the coronate larva of brooding species, which lacks both shells and digestive tract and is obligatorily lecithotrophic (Zimmer & Woollacott, 1977a).

Following larval settlement on a suitable substratum, the larval structure is broken down and the larva metamorphoses into a primary zooid or ancestrula (Zimmer & Woollacott, 1977b). The ancestrula has a feeding polypide, but may be morphologically very different from later autozooids (Ryland & Hayward, 1977). It gives rise to a number of daughter zooids, often in a species-specific order and/or pattern. The process of colony formation is referred to as "astogeny" (derived from Greek *asty* = town), as opposed to "ontogeny", which in bryozoological terms is reserved for zooid ontogeny.

## 1.2 Modular organisation and its consequences

The concept of modular organisation is rather recent in biology. Originally a technical expression used to denote a repeated structure in architecture, the term "module" (*sensu* Chapman, 1981) now is being widely used in biology to refer to a multicellular unit of construction. Modular organisms arise from a zygote, producing a primary module which then itself generates further generations of similar or identical modules. The Bryozoa can be considered as fully modular within the above definition.

Modularity has evolved many times; most plants are modular, as are some protists and fungi and no less than 19 phyla of animals (Begon *et al.*,1990). Animal colonies (*sensu* Hughes1990) are modular, but some authors refer to them as clones of modules.

A number of important advantages are linked with modular construction (Hughes, 1990): the clonal nature of the colony and the asexual replication of modules mean that senescence typically can be markedly delayed or even precluded. The generally small size of modules is crucial in circumventing the allometric relationship between metabolic rate and body mass, which results in a higher metabolic rate and thence rapid development and reproduction. Survivorship of the genome of a modular colony is increased by the fact that the risk of overall mortality is spread between modules. Modularity also allows great plasticity of growth form and brings about high regenerative capacity, the latter being of

particular importance for sedentary organisms that are immobile and have no means of escaping physical or biological disturbances.

Amongst marine fouling assemblages, colonial animals often outcompete solitary animals in competition for space because their indeterminate growth allows continuous lateral expansion and because they are less susceptible to fouling and overgrowth (Jackson, 1977). Greater biodiversity in colonial species is brought about by spatial competition mechanisms (e.g. allelopathy), zonation patterns and preferential settlement of larvae. Recruit mortality is generally lower than in solitary fouling organisms, since the latter are typically opportunistic, generalist species that settle in the early stages of community succession and are therefore more prone to predation effects, whereas colonial species are late settlers and more specialized (Jackson, 1977). Reduced recruit mortality in colonial species is, however, balanced by the fact that a considerable part of their resources is invested in spatial competition mechanisms and hence not available for reproduction.

The degree of integration of modules in colonies varies markedly between phyla and between species within phyla (Harper *et al.*,1986). Depending on its environment, a modular organism will be selectively favoured when its modules are maximally integrated, as in for example siphonophores, or when module individuality prevails as in *Hydra*; between these extremes, a continuum of strategies can be found.

Somatic mutations - *i.e.* mutations that affect individual modules - can be a major evolutionary force (Slatkin, 1984); organisms that have multiple sets of reproductive organs - as seen amongst bryozoans - have the potential for intracolonial genetic variation through the accumulation of mutations which will then be passed on as individual modules reproduce. Predictive models of the effects of somatic mutations depend strongly on the frequency of somatic *versus* gametic mutations; if the relative somatic mutation rate is the higher, then somatic mutations will be of greater evolutionary significance than will mutations occurring in gametes (Slatkin, 1984).

## 1.3 GENERAL METHODOLOGY

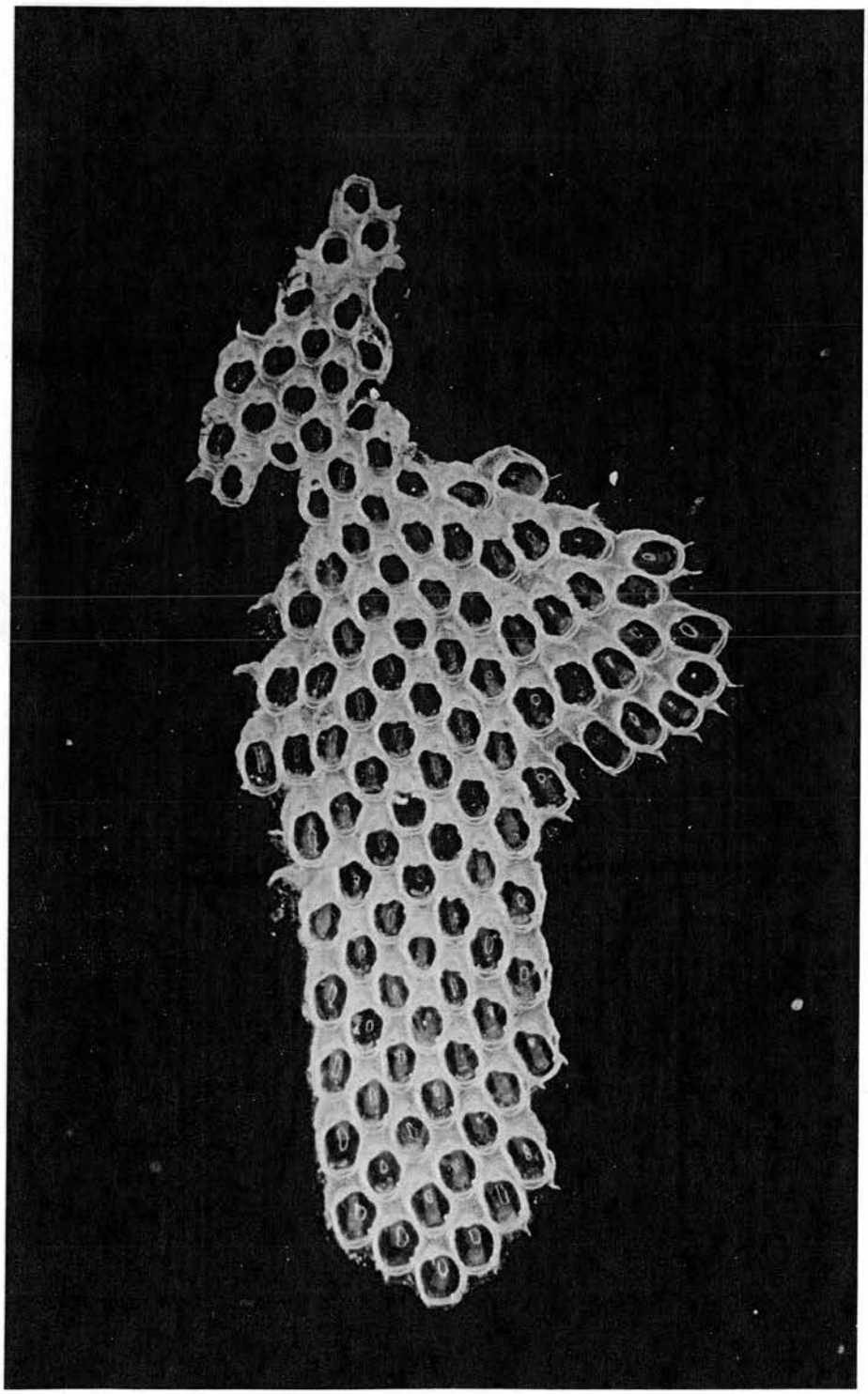
### 1.3.1 EXPERIMENTAL ANIMAL

*Electra pilosa* (L.) (Cheilostomata: Anasca) is abundant and ecologically important amongst epifaunal assemblages around the British Isles. It exploits a variety of substrata, including rock surfaces, mollusc shells and stones, but is most prevalent on macroalgae such as *Laminaria* spp. and *Fucus serratus* (L.) (Ryland & Hayward, 1977) (Fig.1.3). Its vertical distribution ranges from the intertidal to depths of 50 m or more.

Autozooids in *E. pilosa* are typically oval, with a calcified gymnocyst that covers about half of the frontal area of the zooid, and which is perforated by numerous pores. The opesia is surrounded by slender, slightly curved spines that vary in number between 4 and 12 (Ryland & Hayward, 1977). The median proximal spine can be greatly extended. Unlike many other bryozoan species, polymorphism at the zooid level in *E. pilosa* is restricted to two types of zooids, feeding autozooids and dietellae, or pore chambers, which are considered to be a type of kenozooid (= greatly reduced heterozooids without polypides and often without an operculum).

Growth of *E. pilosa* is typically two-dimensional ("sheet-like") and frontal budding does not occur. Colony form may be more or less circular, but more typically the colony develops a digitate or stellate appearance. Erect growth is not, however, unusual for either colony parts or even whole

Fig. 1.3: Young colony of *Electra pilosa*, photographed on *Fucus serratus* (L.). Colony dimensions approx. 10 x 5 mm.



colonies; there has been some discussion as to whether the erect morph constitutes a separate species or merely an ecophenotypic variant of *E. pilosa* (Norman, 1894; Bobin & Prenant, 1960).

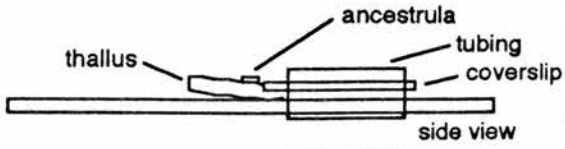
*E. pilosa* is a protandric hermaphrodite, reproducing throughout much of the year, with the exception of late winter (Ryland, 1967; Todd & Turner, 1986). Sperm discharge is effected directly into the water column through pores at the tips of two dorsomedial tentacles of the lophophore (Silén, 1972). The mechanism of fertilization in this species has not been reliably documented. Bonnevie (1907) claimed that fertilization in *E. pilosa* (then *Membranipora pilosa*) was internal, immediately following ovulation, but failed to provide detailed evidence. Marcus (1926) believed fertilization to be internal because spermatozoa placed near ova failed to achieve fertilization success in sea water. Silen (1966) did not specify a mechanism for *E. pilosa* but argued that its congener *E. posidoniae* fertilized externally whilst *E. crustulenta* appeared to fertilize internally, with the intertentacular organ acting as a *receptaculum seminis.* Self-fertilization has not been reported for this species; it appears, however, that selfing is not unusual in gymnolaemates, with a number of both ctenostomate and cheilostomate species displaying this ability (Maturo, 1991).

Egg development in *E. pilosa* is external and results in a planktotrophic cyphonautes larva (Fig. 1.1 B); generally, planktotrophic cyphonautes are assumed to be pelagic for up to two months (Atkins, 1955a). At settlement, the larvae of *E. pilosa* measure about 440 μm at the base and about 360 μm in height (Atkins, 1955b). The feeding mechanism of

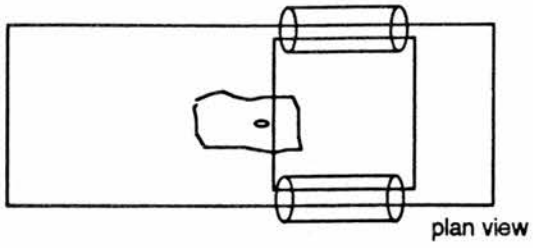
planktotrophic cyphonautes larvae has been described in detail by Atkins (1955a); the cyphonautes produces a continous water current through its mantle cavity by means of a ciliated ridge separating the inhalant from the exhalant chamber. The current delivers food particles directly to the mouth, situated at the end of the inhalant chamber. The alimentary tract is of relatively simple organisation and not substantially different from that of a polypide in an autozooid. Active locomotion in the cyphonautes is achieved by means of a ciliated corona which also supports the current from the exhalant chamber.

During metamorphosis, the larval organization is broken down completely, the ancestrula results, and this subsequently gives rise to the colony. The early stages of astogeny (= colony development) in *E. pilosa* appear to be strictly deterministic in that the pattern of the 4 zooids budded from the ancestrula follows an obligatory sequence (Silén, 1987).

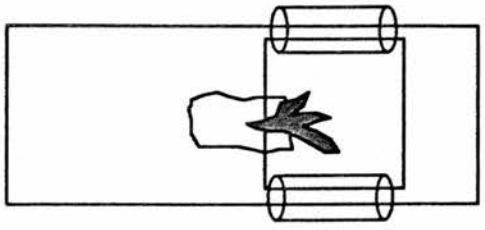
Fig. 1.2: Schematic Representation of Cloning Methodology



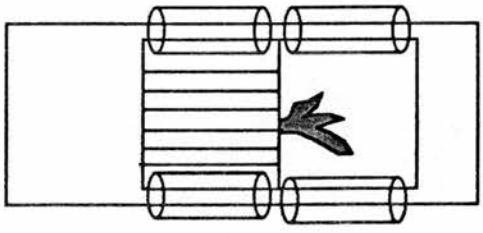

1. Ancestrula on a piece of algal thallus is clipped onto a microscopic slide by means of a coverslip and pieces of PVC tubing.



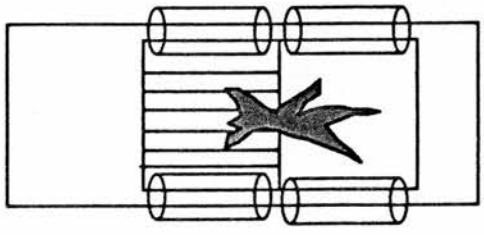

2. The young colony grows from the algal thallus onto the coverslip.



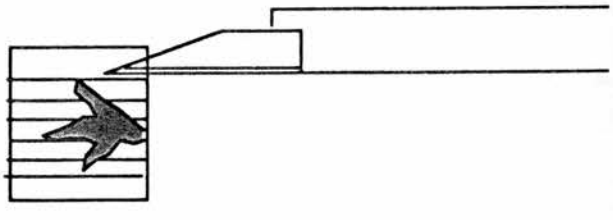
3. The algal thallus is cut off and the prescored coverslip juxtaposed.



4. The colony grows onto the prescored coverslip.

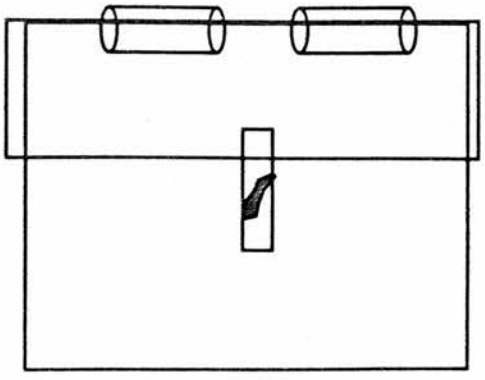


5. The coverslip can be cut up into individual strips with parts of the colony attached ("replication strips").

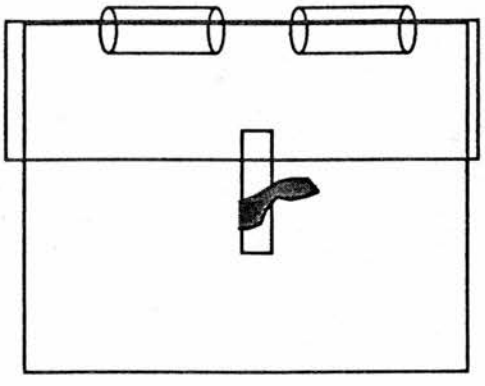


6. Replication strips can be used to grow replicate colonies of the same genotype.

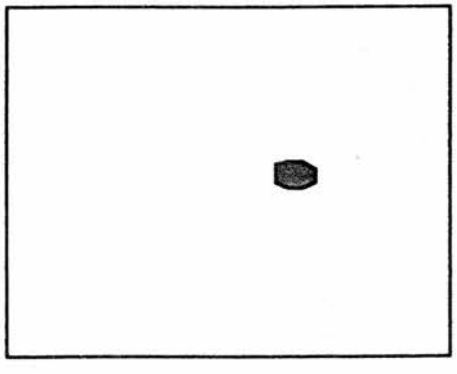
7. The replication strip is clipped onto a glass plate by means of a glass slide and pieces of tubing.



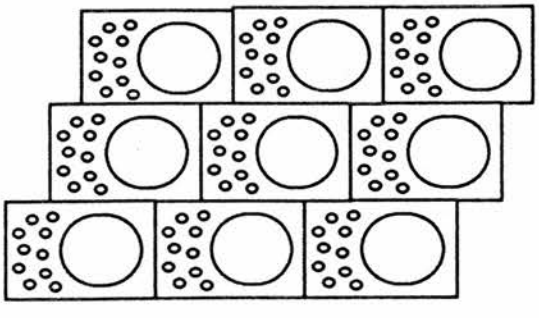
8. The colony grows from the replication strip onto the glass plate.



9. With the colony firmly attached, clamp and replication strip can be removed.



10. The colony is cut back to a standardized starter cluster of several zooids.



11. Colony ongrown for experiment.

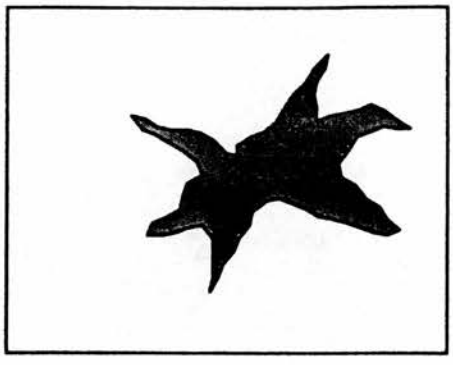
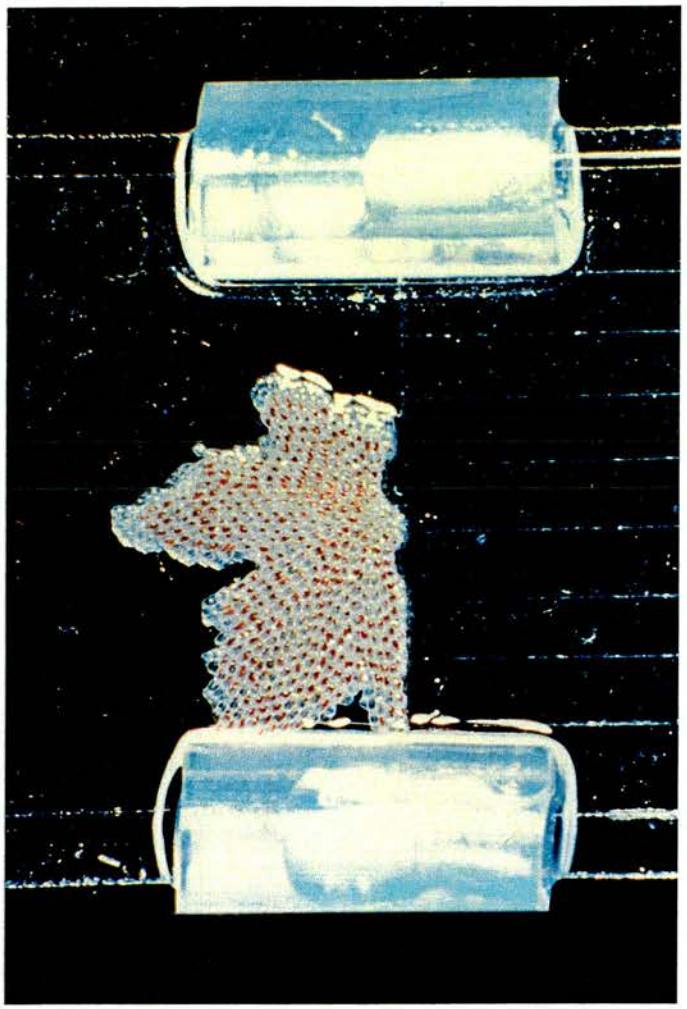


Fig. 1.5: A colony of *E. pilosa*, grown on a microscopic coverslip and juxtaposed with a prescored coverslip which it is about to grow onto (photograph corresponds to step 3 in Fig. 1.2). Note the red colouration of the digestive tracts of individual zooids which is caused by accumulation of the flagellate *Rhodomonas sp.*.



### 1.3.2 CLONING METHODOLOGY

Genetic components of variation were the focus of interest of this study. Bryozoans, like other modular organisms, are extremely well suited for studies investigating genetic variation, since they allow the experimenter to subject ramets of the same genetic individual to different treatments at the same time. Replication of genotypes in bryozoans is a rather simple and inexpensive technique which is now increasingly used in studies pertaining to questions in ecology, evolution and genetics (e.g. Hughes, 1989,1992; Jebram & Rummert, 1978; Hughes & Hughes, 1986a; Jebram, 1980; etc.). However, unlike most species employed in comparable studies, *E. pilosa* is a non-brooding strategist. This has implications for the initial stage of the cloning process, because larvae cannot easily be obtained and used for direct settlement onto the experimental substratum. The problem can, however, be circumvented by using young colonies and permitting them to grow onto experimental substrata as part of their natural growth process. Fig.1.4 provides a graphic representation of the cloning process.

The term "genotype" will be used throughout the text to refer to a genetic individual (synonymous to "genet" or "clone" in the terminology of other authors). This comprises the original colony or ancestrula collected from the field and all derivatives thereof; *i.e.* replicates obtained from the cloning process described below. The assumption of genetic individuality can be made due to the fact that larval production is sexual, and individual colonies will at the most be

full sibs. Fragmentation has not been reported for *E. pilosa*; however, even in the case of a colony breaking up, original colony - fragment status can easily be established by identifying the ancestrula. The deterministic budding pattern in early astogeny of *E. pilosa* lends itself to reliable identification of the latter.

A small piece of the thallus of a *Fucus serratus* plant bearing an ancestrula in the post-metamorphic stage (with no zooids budded) is excised and clipped onto a 76 x 24 mm glass microscope slide, using a glass coverslip and pieces of slit polyvinylchloride tubing as clamps to secure the slips to the slide surface (Fig. 1.4, step 1). Colony growth onto the coverslip is facilitated by removal of the upper epithelial layers of the alga adjacent to the bryozoan margin and clamping of the slip onto the resectioned algal frond. This treatment of the alga provides a more or less flush surface between the thallus and the coverslip. Once the colony growing margin has become established on the coverslip (Fig.1.4, step 2), the alga and the remainder of the colony can be excised and discarded. The further propagation of the genotype involves only the ongrowing and subdivision of the zooids attached to the coverslip. In order to obtain genetically identical clones, the colony then is allowed to grow off the initial coverslip and onto an abutting pre-scored clamped coverslip (Fig. 1.4, step 3 and 4; s. also Fig.1.5). Again, once the colony has grown onto the pre-scored coverslip, the colony on the original slip can be excised and the replication strips can be broken easily with a scalpel blade (Fig.1.4, step 5), each bearing an attached piece of the colony.

These replicate pieces (Fig.1.4, step 6) can then be ongrown onto the definitive experimental glass plates. The replication strip is attached to the glass plate by means of a standard microscopic slide and pieces of tubing (Fig.1.4, step 7), allowing the colony fragment on the replication strip to grow onto the plate (Fig.1.4, step 8). Once a number of zooids have been laid down on the plate, both the clamp and the replication strip can be removed (Fig.1.4, step 9). In order to standardize the starting conditions for all colonies in an actual experiment, it was deemed necessary to remove excess zooids and to reduce the colony to a starter cluster (Fig.1.4, step 10) of a fixed number of zooids (usually 9 or 12). Regeneration of the zooids injured in the process is rapid, usually requiring several days, after which colony growth proceeds in a normal fashion.

The possibility of artefacts arising from the propagation method described above can be confidently dismissed, since all experiments carried out in the course of the study yielded consistent responses for all replicate colonies of a given clone. Also, starter clusters were determined in such a way that the arrangement of zooids relative to each other was similar for all colonies, resulting in equal numbers of free budding loci. Approximately two months are required to obtain the experimental colonies by this propagation method.

### 1.3.3 GENERAL CONDITIONS FOR CULTURE OF *E. PILOSA*

To prevent faeces and microalgae from fouling the experimental colony surfaces, the glass plates in the tanks were held in a vertical orientation by means of perspex racks. Colonies also were cleaned of algal debris on alternate days

during and between experiments, using a soft artist's paintbrush. Apparently, this did not harm the colonies in any way and guaranteed unhindered evagination of polypides at all times.

As a result of the collection of the original colonies from the field, and the subsequent cloning process, introduction of other organisms into the culture tanks could not be avoided. Both stalked and free-living ciliates were present in the cultures at all times, as well as an unidentified nematode species. Also, fouling by diatoms occurred regularly. The abundances of these organisms were, however, closely constrained by regular water changes and manual cleaning of the colonies, and there did not appear to be any effect of the presence of these organisms on the bryozoans. Centrally positioned airstones provided even aeration and water flow between all glass plates and enhanced both algal cell availability and reduced the tendency of the colonies to become fouled.

The culture temperature chosen for both the experimental and between-experimental phases was 18° C. This is high for a boreo-arctic species, but sea surface temperatures in St.Andrews Bay regularly attain 16° C at Bell Rock in the summer months (data obtained from Meteorological Office, for 1990 and 1991). Routine data collated by staff from the Gatty Marine Laboratory showed a temperature range of 3.8-16°C for 1993 (Kinkell Braes, St. Andrews Bay).

Following collection, colonies were acclimated to the culture temperature in daily increments of 3°C. Experimental

temperature was controlled by placing the culture tanks in a water bath, at18°C ; this was itself maintained in a constant temperature coldroom (10° C). The temperature in the tanks was monitored on a daily basis throughout the experimental and observational period.

The bryozoans were cultured in UV-irradiated 20 μm-filtered sea water, which, generally, was replaced every seven days (with the exception of experiment 1).

1.3.4 DIET

The food source chosen for this study was the flagellate cryptophyte *Rhodomonas* sp. (isolate obtained from the NERC Culture Collection of Algae and Protozoa, strain number CCAP 995/2), which has proved to be a satisfactory monospecific diet for bryozoan culture (Kitamura & Hirayama, 1984; Hunter & Hughes, 1991,1993). Dietary quality has been shown to have some effect both on growth and form in bryozoans (Winston, 1976; Jebram, 1980; Jebram & Rummert, 1978); a comparison of different diets could, however, not be achieved in the present study because of time constraints. Nonetheless, the efficacy of *Rhodomonas* for rearing numerous other marine invertebrates and larvae indicated the suitability of this cryptophyte.

Following preliminary trials, Provasoli's Enriched Seawater (Provasoli, 1968) was selected as the culture medium; Provasoli's medium was, however, found to support maximal growth of *Rhodomonas* when nitrate levels were doubled to 166 $mg \cdot l^{-1}$.

*Rhodomonas* sp. was grown in batch culture in 3-l Erlenmeyer flasks. Thorough aeration is critical to microalgal culture, not least because of the risk of $CO_2$ limitation, and was maintained throughout the culturing process. Cultures were grown to an optimal population size of approximately 2500 cells·$\mu l^{-1}$ within 5-6 d, and then were harvested. Cells were separated from the culture medium by centrifugation (1410 g for 10 min.) prior to their use in feeding bryozoans: centrifugation assisted in minimizing growth of both other algae and bacteria in the bryozoan cultures.

The concentration of the resulting *Rhodomonas* suspension was determined immediately before daily feeding by measuring the absorbance of the suspension with a Dynatech MR 5000 photospectrometer. In order to reliably calculate cell number from the absorbance readings, quantification of a test suspension was obtained by counting ten samples on a haemocytometer; a serial dilution then was prepared from the suspension and seawater, with concentrations ranging from 0 to 100%, and every concentration replicated fourfold. Absorbance values were obtained for the dilutions (n=40), which were then regressed on the respective cell counts ($r^2$= 0.995).

All ongrowing experiments required strict control of food concentration, which necessitated daily monitoring of algal concentration in the experimental tanks and then subsequent replenishing. Cell concentrations in the bryozoan cultures were obtained by counting cells in five randomly chosen squares of a Sedgewick-Rafter chamber (sample volume 1 ml, square volume 1 $\mu$l), and calculating their mean. In

experiment 1, the daily change of water in the tanks precluded the necessity of this precaution.

### 1.3.5 ASSESSMENT OF POLYPIDE LIFE SPANS

The advantage of growing *E. pilosa* on glass plates is that zooidal internal features can be observed easily through the transparent basal membrane of the zooids; accordingly, astogeny of the colony was mapped at intervals in order that measures of growth rates, and the daily scoring of polypide de- and regeneration would be routinely achieved using a *camera lucida* mounted on a Wild M8 stereomicroscope. So-called "observational zooids" were identified for routine long-term observation in the case of experiment 1. Observational zooids could easily be reidentified from the *camera lucida* on a day-to-day basis since the spatial arrangement of zooids in the observational cluster is unique. During experiments, the developmental status of each observational polypide had to be determined every 24 h, because polypide degeneration occurs within only a few hours of the termination of polypide feeding activity.

Polypides were classified as 'degenerating' when ciliary activity in the pylorus region of the stomach had stopped; this is a discrete event and allowed classification of polypide condition without any element of subjectivity. Conversely, polypides were classified as 'intact' (*i.e.* as being in their functional feeding stage) once the brown body of the old polypide had been ejected. This generally occurs during one of the first evaginations of the newly-formed polypide, and was a more reliable criterion than was ciliary activity because the

latter resumes before the first evagination of new polypide. The number of consecutive 'intact' observations before regression was taken as a functional polypide's life span, and is thereafter referred to as such.

### 1.3.6 MEASUREMENT OF COLONY GROWTH AND FORM

Generally, colony outlines were drawn with the *camera lucida* every seven days, and the drawings digitised on a Kontron Videoplan image analyser to provide measures of colony area (A) and perimeter (P). In theory, colony form could be analysed by establishing the fractal dimension of colonies, but a simpler and equally reliable method is to calculate a shape index. Two numerical indices of shape can be calculated from colony area and perimeter measurements: Perimeter-Area Ratio (PAR) = P/A (Hunter & Hughes, 1993), and the dimensionless Relative Colony Perimeter (RCP) = $[P^2/4\pi A]^{1/2}$ (Jebram & Rummert, 1978). These measures both are expected to be greater for stellate colonies, and lesser in more circular or lobate forms. RCP provides the advantage of being dimensionless, which removes the size bias of PAR; PAR decreases with colony size, because perimeter increases to the power of 1, whereas area increases to the power of 2, which results in the index itself decreasing as colonies become bigger. RCP, however, being dimensionless and hence a realistic measure of colony form, increases with colony size, indicating that colonies grow more stellate as they grow bigger. A third measure of colony form is provided by intercept and slope of least squares regressions of ln(perimeter) on ln(area).

Mean relative growth rates of colonies can be calculated as :

$$R = \frac{\ln[\text{final area}] - \ln[\text{initial area}]}{t}$$

where t was the intervening time in d (modified from Causton, 1983). This method of calculating growth rates for the whole of the experimental period smooths temporal variations for a given colony and gives an indication of the overall growth performance of that colony. Additionally, colony area at the end of the experimental period could here be used as a measure of colony growth performance, because all colonies started at the same number of zooids.

Colony area was used, rather than zooid numbers, because it provides a more appropriate measure of biomass, and because zooid counting in big colonies measuring several thousand zooids is prohibitively time-consuming.

### 1.3.7 SAMPLING SITES

Specimens of *E. pilosa* were collected from two sites: Clachan Seil, Argyll, Scotland (56° 17' N : 5° 37' W), a sheltered narrow tidal strait on the west coast of Scotland, and Kinkell Braes, St Andrews Bay, Fife, Scotland (56° 27' N : 2° 58' W), a typical semi-exposed rocky shore on the east coast of Scotland.

# CHAPTER 2

# INFLUENCE OF GENOTYPE AND FOOD CONCENTRATION ON POLYPIDE REGRESSION

## 2.1 INTRODUCTION

An important feature in the context of senescence amongst Bryozoa is the phenomenon of polypide regression. The term is used generally to describe the cycle of ageing, death and replacement of individual polypides within their zooids. Polypide regression, or "brown body formation", occurs throughout the Bryozoa, but has been described in detail only for the class Gymnolaemata (e.g. Römer, 1906; Marcus, 1926; Bronstein, 1938; Gordon, 1973, 1977; Cummings, 1975; Dyrynda, 1981).

Gordon (1977) described polypide regression in the cheilostome *Cryptosula pallasiana* both at the light microscopical and ultrastructural level. In this species, the lophophore and digestive tract of the senescing polypide are broken down actively by a combination of autophagy and heterophagy. Shortly after the onset of the degeneration of the old polypide, a new polypide starts to develop from the tissues of the zooid wall and grows toward the remains of the precursor. Cell debris from the old stomach and caecum is compacted into the so-called "brown body", which is itself then enveloped by the stomach of the newly developing polypide and later ejected during one of its first evaginations. In some

other species the brown bodies are retained within the coelom of the zooid (Gordon, 1977) and have been argued to comprise a form of protection against predation (Harvell,1984).

Dyrynda (1981) described contrasting patterns of polypide regression in the cheilostomes *Epistomia bursaria* and *Chartella papyracea*. *E. bursaria* produces a single generation of polypides per zooid, resulting in a narrow band of active zooids at the colony periphery. Polypides in gonozooids degenerate at a certain distance from the growing edge of the colony. *C. papyracea*, by contrast, is characterized by cyclic polypide regression and regeneration; the species is perennial and, concurrent with the cessation of growth occurring in winter, most polypides degenerate. A new generation of polypides is formed in spring when growth recommences. In both androzooids and gynozooids, polypide cycles and gamete maturation are synchronized, with sperm release and ovulation occurring after every second polypide generation.

Bronstein (1938) described the regression-regeneration cycle of polypides in *Membranipora membranacea*; it was demonstrated that polypide regression could be artificially induced by a variety of factors such as lack of oxygen, toxicants (KCN, alcohol), and enforced extended periods of evagination. With adverse environmental conditions persisting, new polypides continue to be formed but degenerate before they start feeding, until after four or five cycles the zooid dies off itself. Bronstein also showed that removing the polypide from the zooid induces the formation of a new bud, and concluded that the presence of a functional polypide hormonally suppresses bud formation in the zooid.

Römer (1906) carried out a detailed histological examination of polypide degeneration and regeneration in *Bugula avicularia* and *Alcyonidium mytili*. He also reported on the existence of zonation patterns in *B. avicularia*: zooids at the colony periphery contained mostly functional polypides whilst the more proximal areas were characterized by functional and degenerating polypides. Zooids at the colony centre contained almost exclusively degenerated polypides. Römer described the degeneration process as comparatively rapid, as indicated by the low incidence of polypides in early degenerational stages. Brown bodies and regenerated polypides never occurred simultaneously, from which he concluded that the brown body must be used for the regeneration of the new polypide, rather than being defaecated as described later by Gordon (1977) for *Cryoptosula pallasiana*. Römer also suggested that increased mechanical stress on the polypide caused by oocyte, and later embryonic, development might induce polypide regression in *A. mytili*.

Although brown bodies had been identified as senescence products as early as the 18th century (Ellis, 1755 [quoted by Gordon, 1977]), the phenomenon has so far been subject to little experimental investigation. Marcus (1926) was the first to demonstrate that brown body formation could be induced experimentally in *E. pilosa*, namely by exposure of colonies to adverse environmental conditions such as changes in salinity, temperature extremes, and lack of oxygen or food. In other species factors inducing polypide degeneration include sexual maturation and/or brooding of larvae, and competitive overgrowth of colony parts (Hyman, 1959; Ryland, 1970).

Cummings (1975) reported widespread polypide degeneration in colonies of *Schizoporella unicornis floridana* at the onset of autumn, with their subsequent regeneration in spring. Polypide degeneration and regeneration in certain species may, however, occur regularly in the absence of external stimuli (Gordon, 1977), although the regulatory mechanism has yet to be established. It has been suggested that the accumulation of metabolic products in the alimentary tract might induce the degeneration of the polypide, and a number of authors have addressed the phenomenon in the context of an excretory function (see review by Gordon, 1977). Ryland (1976) deduced that if the accumulation of residual material in the gut does indeed trigger polypide degeneration, then functional polypide longevity should decrease with an increasing amount of food ingested. In a recent study, Hunter & Hughes (1993) noted a significant effect of food concentration on brown body counts in *Celleporella hyalina*, but their study did not establish actual polypide life spans.

The present study provides the first experimental test of Ryland's hypothesis, by subjecting laboratory-propagated replicate genotypes of *E. pilosa* to different food regimes. Analyses were undertaken on the duration of the life span of the functional feeding polypide. Additional analyses were undertaken for data relating to whole-colony growth rate and shape. Colony growth rate is an important fitness-related trait, because fecundity in bryozoans generally is directly proportional to colony size (Thorpe, 1979). Studies of phenotypic plasticity of colony form have been carried out on various species' responses to a number of environmental

variables (e.g. Winston, 1976; Jebram & Rummert, 1978; Jebram, 1980; Okamura,1992; Hunter & Hughes, 1993), but at present there are no quantitative studies of the effects of diet on colony form in *E. pilosa*.

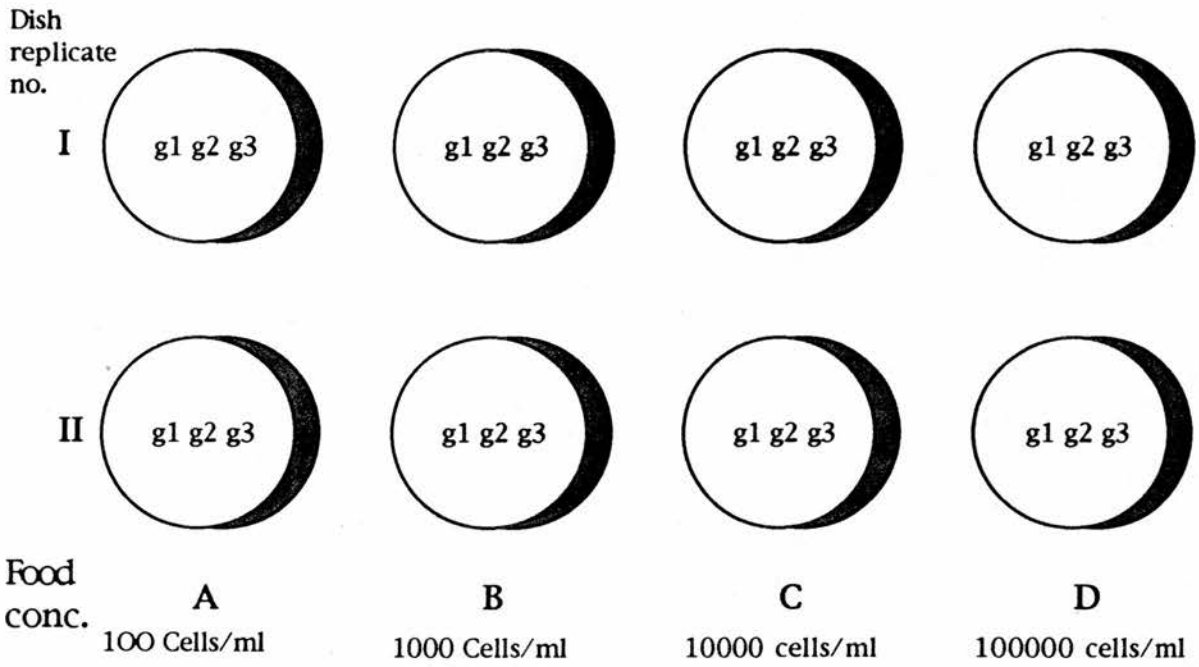
## 2.2 MATERIALS AND METHODS

### 2.2.1 EXPERIMENTAL SET-UP

Prior to the experiment, young colonies of *E. pilosa* (approximately 10-100 zooids each) were collected *ad hoc* from *Fucus serratus* fronds at Clachan Seil and St Andrews Bay in January 1992. Colonies were acclimated and replicated as described in section 1.3.

For the purposes of this experiment, eight replication strips of each of three randomly chosen genotypes (genotypes 1 and 3 from St. Andrews Bay, genotype 2 from Clachan Seil) were obtained. Each of the 24 replicate strips was clipped to individual 50 x 60 mm glass plates, onto which the colonies grew quickly. At the start of the experiment, each colony was reduced by excision to a cluster of 12 zooids each. In order to preclude any possible adverse effects on the cluster of 12, of the process of cutting away excess zooids, the first 20 zooids budded by that cluster were intended to be used for the monitoring of polypide regression ("observational zooids"), rather than using the cluster itself.

Fig. 2.1: Arrangement of colonies in tanks. "g1", "g2" and "g3" represent replicates of the respective genotypes. Roman numerals denote replicate dishes, capital letters denote different treatments.




Colonies then were transferred to 8 circular 1-l glass tanks, with one replicate of each of the three genotypes per tank (Fig.2.1). The arrangement of colonies in the tanks did not require randomization, because there was only one possible configuration of the three genotypes.

Tanks were maintained at 4 different food concentrations ($1x10^2$ cells·ml$^{-1}$, $1x10^3$ cells·ml$^{-1}$, $1x10^4$ cells·ml$^{-1}$, $1x10^5$ cells·ml$^{-1}$), with duplicate tanks at each concentration. Due to feasibility constraints, the extent of replication had to be restricted to duplicates.

Colony area measurements were obtained every 7 d. All three measures of colony form (RCP, PAR, regression coefficients) were calculated from colony area and perimeter

measurements. Colony growth was analyzed on the basis of mean relative growth rates over the whole experimental period. Polypide status in the observational zooids was established every day. The experiment was carried out for a duration of 48 d in February and March 1993.

### 2.2.2 STATISTICAL ANALYSIS

Although observations were made for individual identifiable zooids within each colony, zooids are unlikely to be independent within a colony because of the funicular connexions between zooids (Ryland, 1979). Because the focus of the present study was on the whole colony response to food concentration, the relevant analytical data are the average responses of colonies. The patterns of dependence between individual zooids is to date not understood and will be explored elsewhere (Bayer *et al.*, in preparation).

At the colony level the experiment had a split-plot design, with food concentration as a whole-plot (*i.e.* tank) treatment and genotype as a within-tank factor. The appropriate analysis of variance is described by Snedecor & Cochran (1967) and was implemented in GLIM (Payne, 1987).

The data were examined first for outliers, and the Box-Cox methodology (see e.g. Aitkin *et al.*, 1988, Ch. 3) was used to determine whether or not the data required transformation in order to satisfy the necessary assumptions of equal variances, additivity of tank, food and genotype effects, and normal distributions.

2.2.2.i Polypide life span

Zooids were followed through up to three complete cycles of polypide regression and regeneration in given colonies. The three cycles give repeated measures on each colony (nb that the term 'cycle' in this context refers to the functional polypide's life span in the respective cycle, not the duration of the whole cycle). Although not the ideal form of analysis (Mead, 1988, Section 14.5), the repeated measure factor was treated as another factor at the split-plot level, having possible interactions with food and genotype. For simplicity the results presented are from these analyses, but they were confirmed by analyses of data from each cycle separately and by a further analysis of the change between the first and third cycle.

Because of unequal timing of the initiation of zooids, the occurrence of resting phases, and censoring of data (*i.e.* observation ceasing before the third polypide generation had started regressing) different numbers of life spans were available to form average colony life spans. This suggests that colony life spans might be given different weights. However, because of the lack of independence between individual zooids, weighting by the number of active zooids will overcorrect. Both a weighted and an unweighted analysis were carried out. Censored life spans were considered separately.

2.2.2.ii Colony Form

PAR and RCP were analysed in the same way as mean relative colony growth rates and polypide life spans.

Both were $\log_{10}$ transformed prior to analysis, as indicated by the Box-Cox procedure. Shape *per se* was studied by investigating how the intercept and slope of least squares regressions of ln(perimeter) on ln(area) depended on genotype and food level.

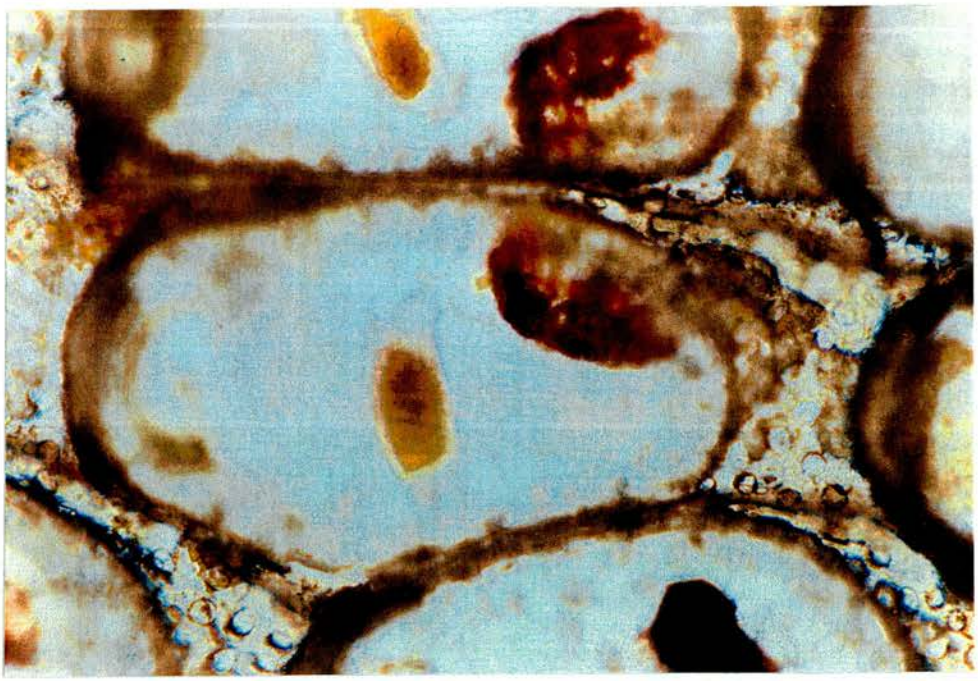
## 2.3 RESULTS

### 2.3.1 POLYPIDE LIFE SPANS

Polypide regression in *E. pilosa* followed the same pattern as described by Gordon (1977) for the cheilostome *Cryptosula pallasiana*. Degeneration starts at the lophophore and the rectum of the polypide, with the proximal parts ensuing. In the early stage of brown body formation, the bipartite appearance of the polypide remains is clearly visible, with lophophore, rectum and pharynx remains forming the upper, lighter part of the debris mass, and stomach and caecum forming the condensed, darker part (Fig. 2.2.3). While the debris mass is being condensed further, a polypide primordium develops from the distal zooid wall, growing towards the forming brown body, eventually enveloping it in what will develop into the stomach of the new polypide (Figs. 2.2.4, 2.2.5). Condensation of the brown body proceeds until the lighter parts are finally broken down, and only the remains of the old stomach are left. After the completion of the development of the new polypide, the brown body is defaecated during one of the first lophophore evaginations.

Fig. 2.2.1 (top): Early stage of a feeding polypide. The red colouration of stomach, caecum and rectum is a consequence of the accumulation of pigments from the food source, *Rhodomonas sp.*.

Fig. 2.2.2 (bottom): Late stage of feeding polypide. Colouration of digestive tract is now clearly darker than in Fig. 2.2.1, a result of the increasing accumulation of waste products in the epithelial cells.



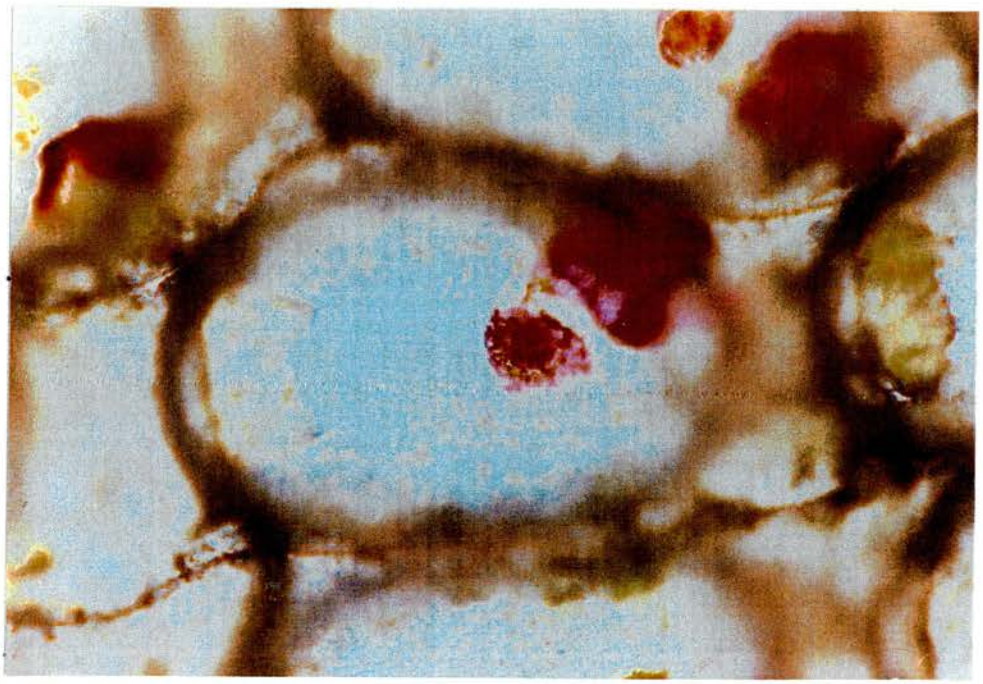
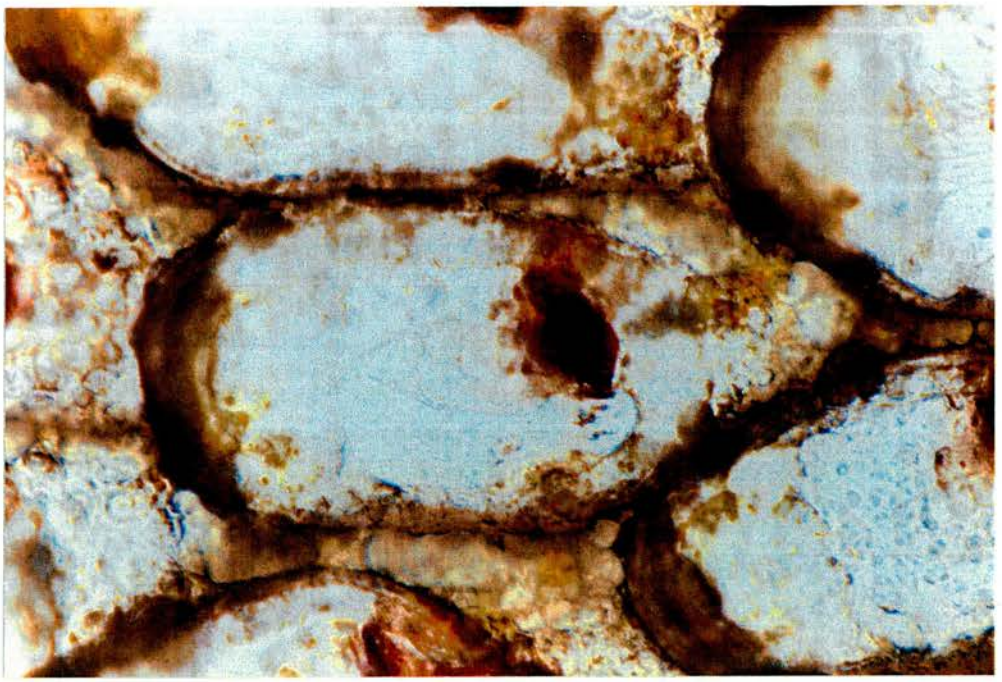


Fig. 2.2.3 (top): Regressing polypide, shortly after the onset of degeneration. The bipartite appearance is presumably caused by lophophore/pharynx/rectum and stomach/caecum regressing as two rather separate units (cf. Gordon, 1977).

Fig. 2.2.4 (bottom): Early stage in the regeneration process. The remains of the old polypide have been compacted further, and a new polypide is being formed from distally, growing towards the brown body.



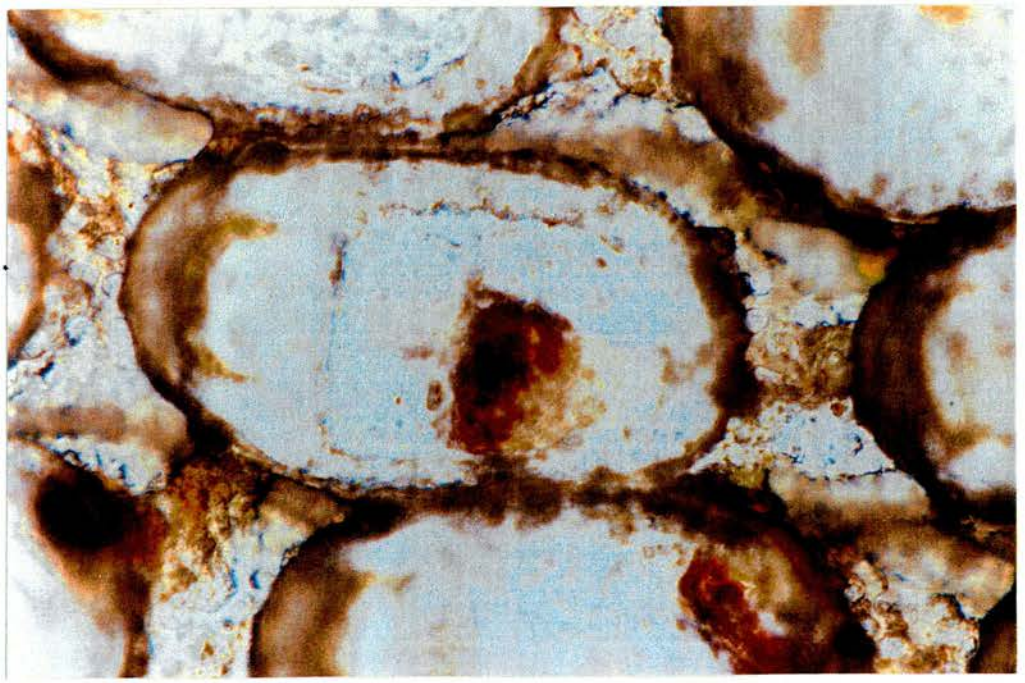
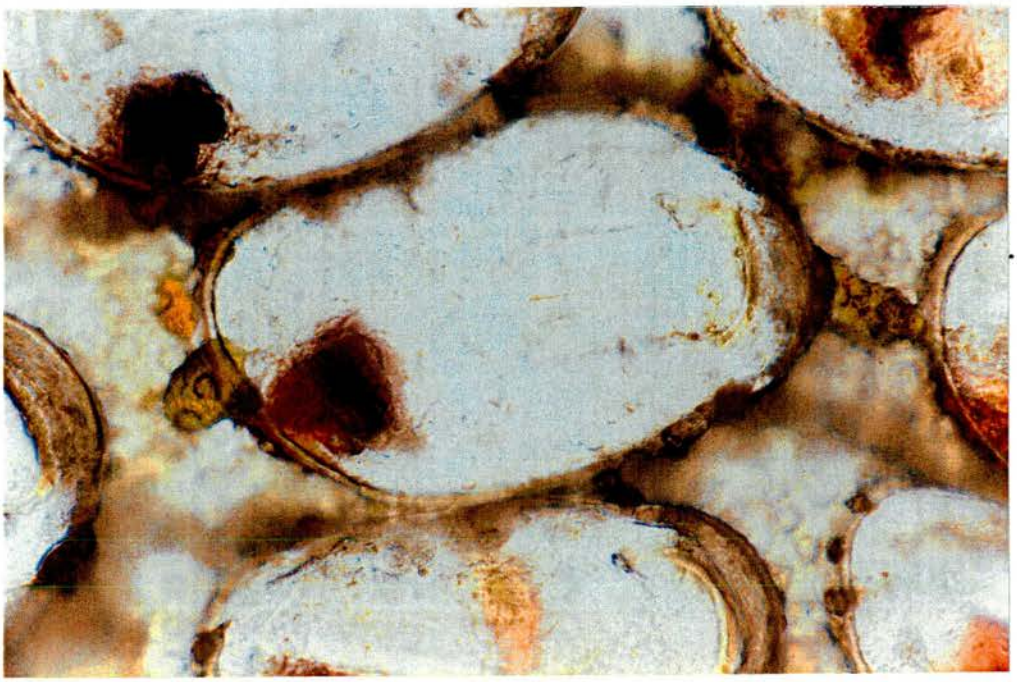


Fig. 2.2.5: Formation of the new polypide. Tentacles are clearly distinguishable now, but have not reached their full length yet. The brown body is in the process of being envelopped by the stomach and caecum of the new polypide; it will then be defaecated during one of the first evaginations.



With a red flagellate such as *Rhodomonas* as food source, the accumulation of waste products in the stomach epithelium is evident by the colour change of the stomach from a light pink in the early stages (Fig. 2.2.1) to a dark brown immediately before the degeneration of the polypide (Fig. 2.2.2).

Towards the end of the experiment, numerous zooids were observed to remain intact (retaining the frontal membrane, operculum, and parietal musculature) but in a 'resting' stage after polypide degeneration. Although a small proportion of these eventually did regenerate new polypides, the majority remained in the resting stage. This probably is indicative of zooid senescence, with zooid death generally ensuing. These stages were allowed for in analysing the data (see below).

At the lowest food concentration investigated ($1x10^2$ cells·ml$^{-1}$), colonies failed to produce a sufficient number of new zooids to provide the data required. The polypide life span data analysed therefore include only the three upper food concentrations. At $1x10^3$ cells·ml$^{-1}$ only genotype 2 budded the required 20 observational zooids: genotypes 1 and 3 budded only between 9 and 11 zooids and hence analytical sample sizes were reduced. In one of the tanks at $1x10^3$ cells·ml$^{-1}$, none of the reduced number of zooids completed the third cycle and the one zooid of genotype 3 completed the second cycle only after an extended period of 18d. This observation was a gross outlier, which led to the Box-Cox procedure indicating a severe transformation, difficult to interpret. If this observation were to be omitted, then no transformation of completed life spans needed to be carried

out for the assumptions of ANOVA to be satisfied. The reduced data set (excluding the outlier and having no transformation) was analysed. In the other tank at $1x10^3$ cells·ml$^{-1}$, genotypes 1 and 3 both budded 8 zooids. For genotype 1 only two of these completed the third cycle; four entered the resting stage after the second cycle and two started the third cycle but were censored after 6 and 7 d respectively.

Unweighted and weighted analyses of completed life spans gave essentially the same conclusions and only the unweighted results are presented. Both food concentration and genotype had highly significant effects on polypide life spans (Table 2.1). Figure 2.3 and Table 2.2 show the decrease of average life span with increasing food concentration and the way in which this differs for the distinct genotypes, as indicated by the significant genotype x food concentration interaction.

The pattern of response to different food concentrations in different cycles was marginally significant, when tested against the polypide cycle x tank interaction, but is not straightforward (Fig.2.3, Table 2.3). At $1x10^5$ cells·ml$^{-1}$, polypide life span remained similar across successive cycles. At $1x10^4$ cells·ml$^{-1}$, life span increased in the third cycle; at $1x10^3$ cells·ml$^{-1}$ (the lowest food concentration permitting analysis of polypide life span), an increase in life span in the second cycle was followed by a decrease in the third cycle. Evidence for this does, however, derive from few available zooids, nearly all of genotype 3. Most of the zooids entering a 'permanent' resting phase at the end of the first (4 zooids) or second (14 zooids) cycle were of genotype 1. These 16

Fig. 2.3: Colony means of polypide life spans at 3 different food concentrations in 3 consecutive regeneration cycles (in days). Error bars are standard errors of replicate zooids from both replicate colonies. Black shading: genotype 1; hatched: genotype 2; white: genotype 3.

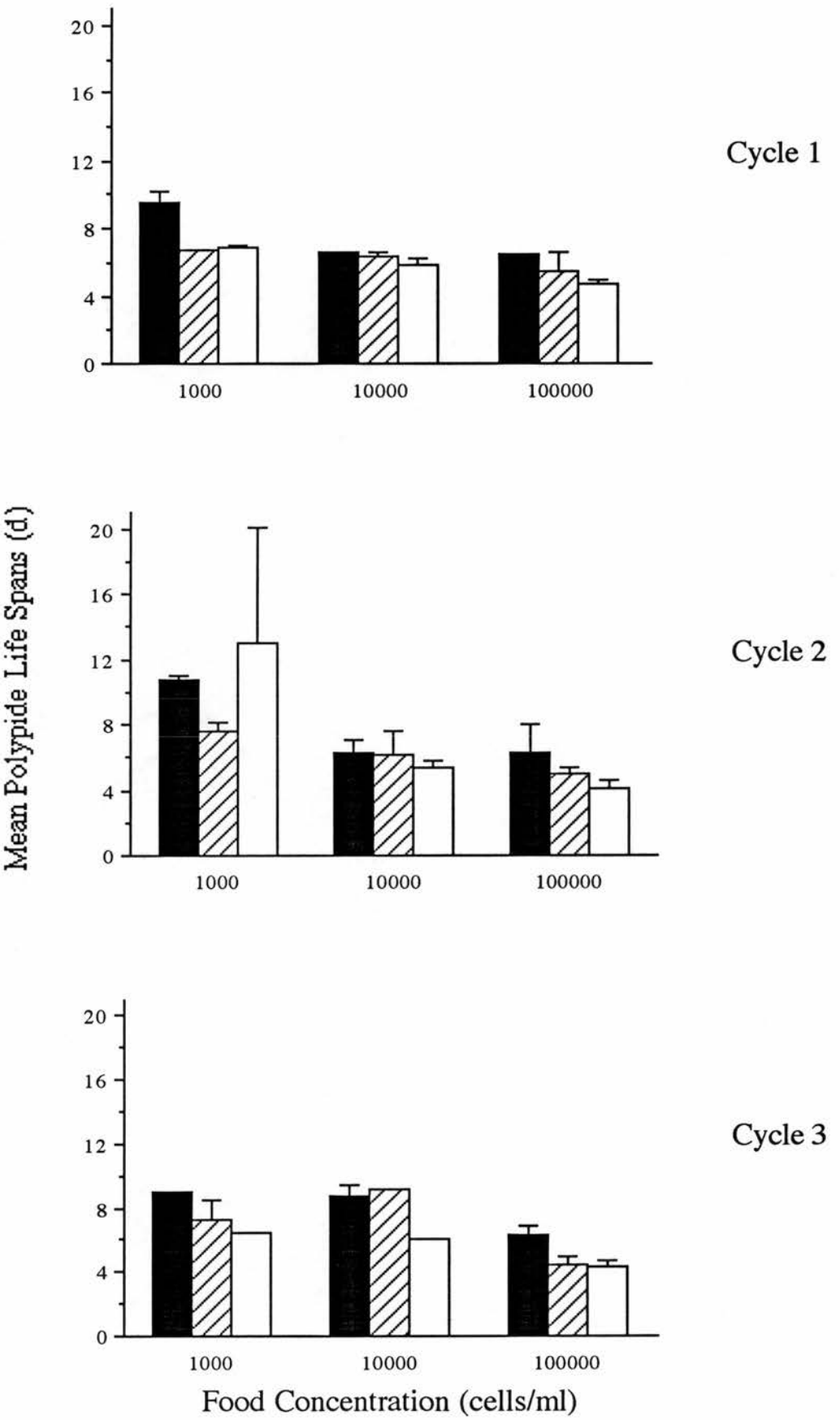

Mean Polypide Life Spans (d)
20
16
12
8
4
0
1000
10000
100000
Cycle 1
Cycle 2
Cycle 3
Food Concentration (cells/ml)

Table 2.1: ANOVA of polypide life spans. Data analysed were colony means from up to 20 replicate zooids per colony. 24 colonies (eight from each of the three genotypes) were used in the experiment, with two tanks at each of the four food concentrations and one colony of each genotype per tank. "Polypide cycle" or "Cycle" refers to repeated polypide generations within the same zooid; the data are from three consecutive cycles.

| Factor | df | SS | MS | *F* | P |
|---|---|---|---|---|---|
| Food Concentration | 2 | 65.210 | 32.610 | 30.00 | 0.01 |
| Tank | 3 | 3.261 | 1.087 | | |
| Genotype | 2 | 36.020 | 18.010 | 55.60 | <0.001 |
| Food x Genotype | 4 | 12.180 | 3.045 | 9.40 | 0.016 |
| Tank x Genotype | 6 | 1.942 | 0.324 | | |
| Polypide Cycle | 2 | 2.295 | 1.147 | 1.36 | 0.34 |
| Food x Cycle | 4 | 15.850 | 3.963 | 4.68 | 0.048 |
| Tank x Cycle | 6 | 5.073 | 0.846 | | |
| Genotype x Cycle | 4 | 1.286 | 0.322 | 1.50 | 0.30 |
| Food x Cycle x Genotype | 8 | 3.980 | 0.498 | 2.32 | 0.16 |
| Residual | 9 | 1.939 | 0.215 | | |
| Total | 50 | 149.040 | | | |

Table 2.2: Colony means of polypide life spans. Data are averaged over cycles and over replicates of genotypes in duplicate tanks, for each of the three food concentrations examined (the lowest food concentration had to be excluded from the analysis because of the lack of observational zooids produced).

| | $1x10^3$ cells·ml$^{-1}$ | $1x10^4$ cells·ml$^{-1}$ | $1x10^5$ cells·ml$^{-1}$ |
|---|---|---|---|
| Genotype 1 | 9.95 | 7.22 | 6.36 |
| Genotype 2 | 7.16 | 7.28 | 4.97 |
| Genotype 3 | 9.21 | 5.75 | 4.40 |

Table 2.3: Colony means of polypide life spans. Data are averaged over all colonies at each food concentration (irrespective of genotype) for each cycle. The lowest food concentration had to be excluded from the analysis because of the lack of observational zooids produced.

| | $1x10^3$ cells·ml$^{-1}$ | $1x10^4$ cells·ml$^{-1}$ | $1x10^5$ cells·ml$^{-1}$ |
|---|---|---|---|
| Cycle 1 | 7.32 | 6.29 | 5.57 |
| Cycle 2 | 8.41 | 5.98 | 5.15 |
| Cycle 3 | 6.95 | 7.98 | 5.01 |

occurred roughly equally amongst the three food concentrations. Because of late initiation, or intermediate resting, 19 zooids entered the third cycle but did not begin regression of the third polypide. Thirteen of these were attributable to one tank, 11 of them being of genotype 3 which otherwise had only one zooid not completing three cycles. Overall seven zooids of genotype 1, and only one of genotype 3, had censored life spans. Because of the problem of non-independence, no modelling of individual zooid life span distribution was attempted, so that the censored data could not be included in a common analysis with the complete data. With the one exception mentioned above, only one or two observations were censored from any colony: their values do not suggest that, had they not been censored, the conclusions would have been altered.

### 2.3.2 COLONY GROWTH

ANOVA of mean relative growth rate of the colonies (R) revealed that there were significant main effects both for food concentration and genotype and that the genotype x food interaction also was significant (Table 2.4). The increase in R with increasing food was rather pronounced over the three lower concentrations investigated, and less so between the $1x10^5$ cells·ml$^{-1}$ and $1x10^6$ cells·ml$^{-1}$ treatments (Fig.2.4). Note that one of the two tanks in the $1x10^5$ cells·ml$^{-1}$ treatment yielded much lower growth rates (asterisked in Fig. 2.4) than did its duplicate, resulting in consistently high variation between the same genotypes in these two tanks. This was due to an inexplicable disruption of growth in the one tank on Day

Fig. 2.4: Mean relative colony growth rates at 4 different food concentrations, for all replicate colonies. Black shading: genotype 1; hatched: genotype 2; white: genotype 3. Asterisks mark tank where temporary disruption of growth occurred.

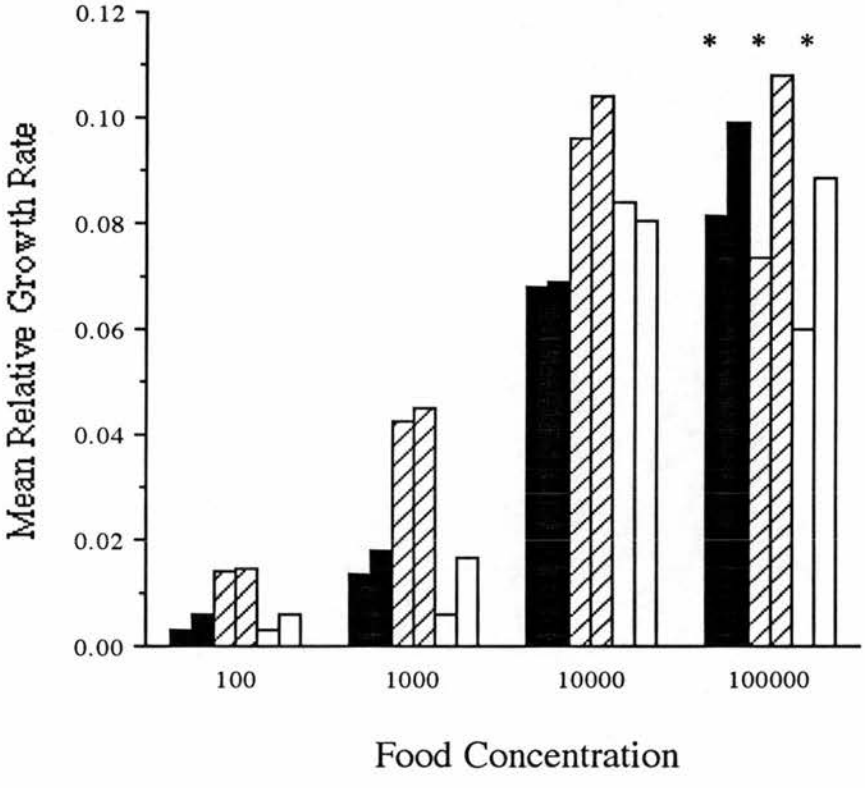

0.12
0.10
0.08
0.06
0.04
0.02
0.00
Mean Relative Growth Rate
* * *
100
1000
10000
100000
Food Concentration

Table 2.4: ANOVA of mean relative growth rates. Data analysed were growth rates of 24 colonies for the whole of the experimental period. Each of the three genotypes was replicated eightfold, with duplicate tanks at each of the four food concentrations, and one colony of each genotype per tank.

| Factor | df | SS | MS | *F* | P |
|---|---|---|---|---|---|
| Food Concentration | 3 | .0291100 | .0097040 | 34.47 | 0.004 |
| Tank | 4 | .0011260 | .0002810 | | |
| Genotype | 2 | .0017780 | .0008890 | 54.20 | < 0.001 |
| Genotype x Food | 6 | .0009204 | .0001530 | 9.32 | 0.004 |
| Genotype x Tank | 8 | .0001312 | .0000164 | | |
| Total | 23 | .0330700 | | | |

Table 2.5: Mean relative growth rates. Data are means of duplicate colonies for every genotype at each of the four food concentrations employed.

| | $1x10^2$ cells·ml$^{-1}$ | $1x10^3$ cells·ml$^{-1}$ | $1x10^4$ cells·ml$^{-1}$ | $1x10^5$ cells·ml$^{-1}$ |
|---|---|---|---|---|
| Genotype 1 | .00443 | .01554 | .06859 | .09022 |
| Genotype 2 | .01406 | .04363 | .10000 | .09070 |
| Genotype 3 | .00450 | .01137 | .08226 | .07420 |

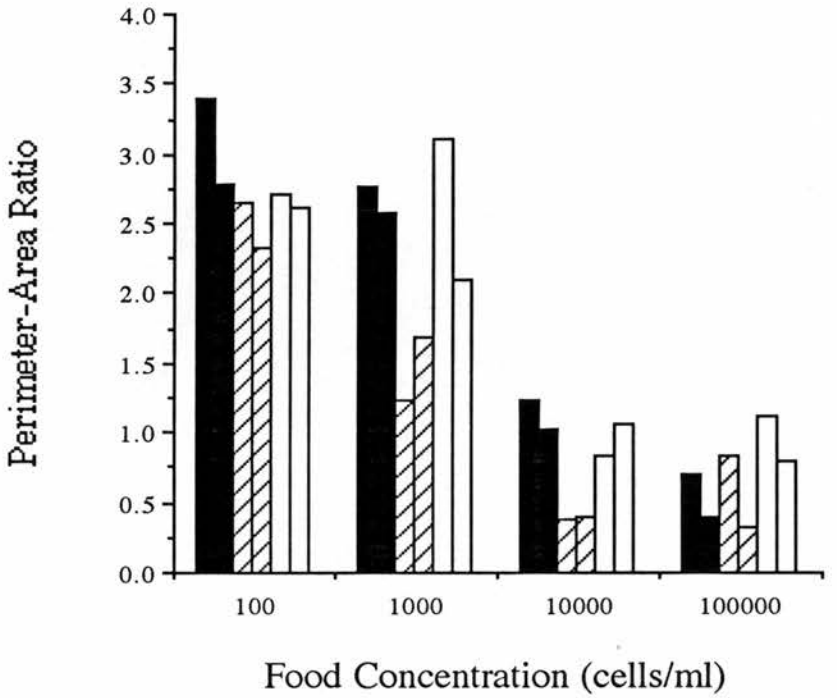
18 of the experiment, which affected all colonies in that tank, and which persisted for several days. Zooidal buds at the growing edge of the colony degenerated suddenly, but resumed growth soon after that. The possibility of some form of interaction between the colonies in the one tank, or an experimental/handling artefact, cannot be ruled out. With the exception of the one anomalous tank, genotype 2 clearly outperformed genotypes 1 and 3 (Fig. 2.4), with the relative discrepancy being greatest at the two lowest food concentrations. Another contributory factor to the interaction is that genotype 3 outperformed genotype 1 at $1x10^4$ cells·ml$^{-1}$, but this growth rate ordination was reversed at $1x10^5$ cells·ml$^{-1}$ (Table 2.5).

### 2.3.3 COLONY FORM

As for R, variations in PAR were attributable to significant main effects and interaction (Table 2.6). For all genotypes PAR decreased over the three lower algal concentrations, and for genotypes 2 and 3 increased slightly between the second highest and the highest food concentration (Fig.2.5). Relative differences of genotype 2 *versus* genotypes 1 and 3 were greatest at the two intermediate concentrations.

The dimensionless coefficient RCP changed significantly only with food concentration (Table 2.7). Genotypes differed, however, to some extent, since genotype 2 did not show any conspicuous differences in RCP between treatments, whereas in genotype 1, the $1x10^4$ cells·ml$^{-1}$ treatment was rather different from the other treatments (Fig. 2.5). For RCP in Genotype 3, the two upper food concentrations

Fig. 2.5: Two different measures of colony form. Top: perimeter-area ratios (PAR). Bottom: dimensionless shape coefficient RCP, calculated as $[P^2/4pA]^{1/2}$. Black shading: genotype 1; hatched: genotype 2; white: genotype 3.


Perimeter-Area Ratio
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
1000
10000
100000
Food Concentration (cells/ml)

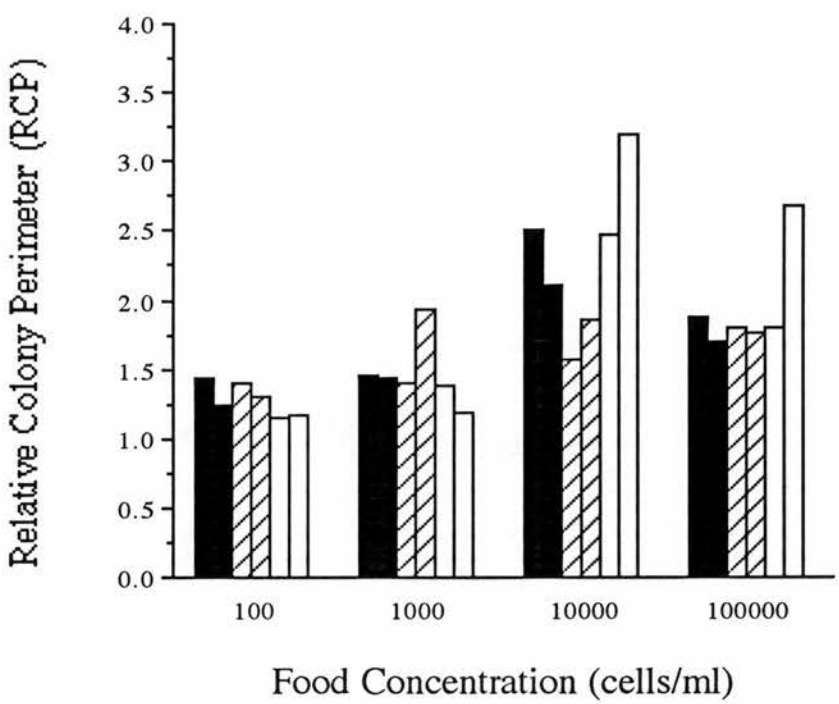

Relative Colony Perimeter (RCP)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
1000
10000
100000
Food Concentration (cells/ml)

Table 2.6: ANOVA of $\log_{10}$ transformed perimeter-area ratios (PAR) of all colonies at the end of the experimental period. Data analysed were from 24 colonies of three genotypes (each replicated eightfold), with duplicate tanks at each of the four food concentrations, and one colony of each genotype per tank.

| Factor | df | SS | MS | *F* | P |
|---|---|---|---|---|---|
| Food | 3 | 9.6080 | 3.2030 | 22.36 | 0.006 |
| Tank | 4 | 0.5730 | 0.1430 | | |
| Genotype | 2 | 1.3340 | 0.6670 | 20.34 | < 0.001 |
| Genotype x Food | 6 | 0.8940 | 0.1490 | 4.54 | 0.03 |
| Genotype x Tank | 8 | 0.2623 | 0.0328 | | |
| Total | 23 | 12.6700 | | | |

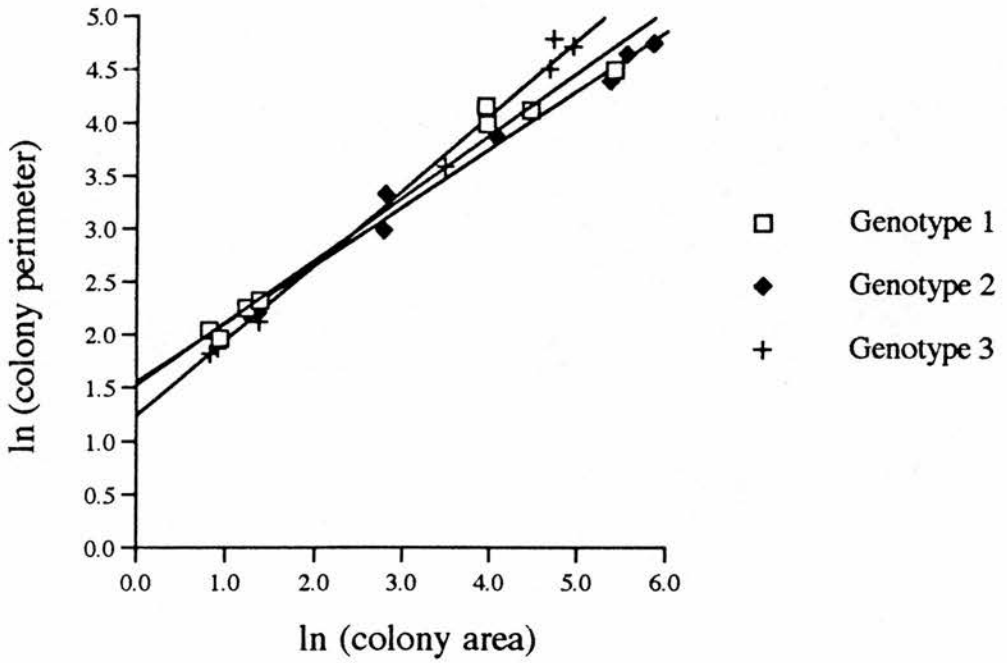
Table 2.7: ANOVA of $\log_{10}$ transformed relative colony perimeters (RCP) of all colonies at the end of the experimental period. Data analysed were from 24 colonies of three genotypes (each replicated eightfold), with duplicate tanks at each of the four food concentrations, and one colony of each genotype per tank.

| Factor | df | SS | MS | *F* | P |
|---|---|---|---|---|---|
| Food | 3 | 1.15100 | 0.383600 | 42.510 | < 0.005 |
| Tank | 4 | 0.03609 | 0.009022 | | |
| Genotype | 2 | 0.02318 | 0.011590 | 0.492 | >> 0.50 |
| Genotype x Food | 6 | 0.38360 | 0.063930 | 2.710 | > 0.10 |
| Genotype x Tank | 8 | 0.18840 | 0.023550 | | |
| Total | 23 | 1.78227 | | | |

differed markedly from the lower ones. It is, however, conceivable that the outcome might have been different had the number of replicates per treatment been higher.

These results are more clearly interpretable on examination of the relationship between perimeter and area (Fig. 2.6). There was evidence of a scaling relationship for each genotype, with perimeter proportional to a power of area: the powers were 0.60, 0.56 and 0.71 for genotypes 1-3 respectively. These are well determined, the two least squares lines, of perimeter on area and *vice versa*, giving estimates differing by 2% or less and the standard error of each being about 0.03. It is evident that absolute colony size plays a major rôle in determining colony shape, but the two measures provide contrasting outcomes because PAR decreases with colony area, whereas RCP slightly increases. As already pointed out in section 1.3 (General Methodology), the size bias in PAR is considerable, and hence the use of this parameter probably produces an artefactual result, whereas the increase of RCP with colony size appears to be real (Hughes & Hughes, 1986b).

Fig. 2.6: Plot of ln transformed perimeter against ln area values of all colonies. Fitted lines represent individual genotypes.


5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ln (colony perimeter)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
ln (colony area)
Genotype 1
Genotype 2
Genotype 3

## 2.4 DISCUSSION

The decrease of polypide life spans with increasing food concentration is strong evidence in support of the hypothesis that the longevity of individual polypides depends on the rate of food uptake; such would concur with Gordon (1977), who concluded that the polypide will degenerate as soon as a certain level of waste product accumulation in the stomach epithelial cells has been reached. It is known from both mammals and invertebrates (e.g cladocerans, planarians, rotifers) that under conditions of submaximal diet longevity can be increased: this has generally been explained by reduced metabolic rates and/or delayed development (Lamb, 1977). None the less, the present data also provide strong evidence for a genetic component to the determination of polypide life spans in *E. pilosa*. Because of the constraints of feasibility the present experimental design had the shortcoming of not providing replicates of given genotypes within the same tank. Despite this, it can be assumed with confidence that the apparent genotypic effect on polypide life spans is real because conditions in the replicate tanks were closely monitored and seemingly identical (with the exception of tank 7). Moreover, inter-colony and inter-genotype variation in this experiment was considerably greater than the variation between individual zooids within a colony.

One underlying assumption in this experiment is that zooids within the one colony are genetically identical, and that feeding conditions do not vary significantly between

zooids in the central (observational) part of a given colony; variation at the zooid level cannot, however, simply be discarded as statistical "noise" because of the possibility of colony-wide, or at least localized, co-ordination of polypide cycles. For example, colony-wide integration has been reported both for the nervous system (e.g. Thorpe *et al.*, 1975) and metabolite transport (e.g. Best & Thorpe, 1985) in bryozoans: if a general phenomenon, it is very likely that at least adjacent zooids will display at least some degree of inter-dependence.

Towards the end of the experiment, certain individual zooids entered a resting stage, which appeared to be a consequence of ageing at the zooidal level, and perhaps a physiological stage preceding zooid death. Both from personal observations and other authors' experience (Hyman, 1959; Cancino & Hughes, 1987), it is apparent that in bryozoan species with multiple polypide generations, zooid death starts to arise at some point in the astogenetic development of the colony, and usually spreads from the proximal to the distal parts of the colony: during this period the younger peripheral zooids remain fully functional and continue to bud. Longevity data for whole colonies or individual zooids are not available for *E. pilosa*. The fact that the central region of the colony generally begins to die after several months under laboratory conditions, and that zooid death is progressive along a proximal-distal gradient, strongly suggest that there must be a maximum life span for individual zooids. The three major possibilities by which zooid longevity could be controlled include:

1) Zooid death is genetically programmed and occurs at a certain ontogenetic zooid age, irrespective of the number of polypide generations that have been produced by that zooid.

2) Zooid senescence is a function of the number of polypide generations produced; there is a genetically and/or physiologically determined maximum number of polypide generations that a zooid can produce, and zooid death will ensue — irrespective of time — after the last cycle has been completed.

3) Higher polypide turnover rates might extend the life span of the living tissue of the cystid and enable it to produce more polypide generations than at lower turnover frequencies. Gordon (1977) has argued that bryozoan polypide regression as such has a rejuvenatory effect on individual zooids, in a manner analogous to the deductions of Toth (1969) for the hydroid *Campanularia flexuosa*.

1) and 2) above are plausible and likely explanations and it is generally agreed that genotype has a major effect on the rate of senescence (e.g. Lamb, 1977; Rinkevich *et al.*,1992); with specific reference to bryozoans, and recognizing the relatively high degree of zooid individuality in cheilostomes (Ryland, 1979), intuitively one would expect any genetic influence on ageing to be expressed at the level of the zooid. For strictly genetically determined zooidal life spans, a high rate of polypide turnover could constitute a selective disadvantage for the colony, because the metabolic cost of producing repeated generations of polypides

would not necessarily be balanced by any benefit in resource acquisition.

If there were a maximum number of polypide generations that a zooid could produce, then faster turnover of polypides, as observed at the higher food concentrations in the present experiment, would result in earlier death of the zooid; this, again, might be disadvantageous for the colony. Growth rates vary significantly between genotypes (see also Chapter 3), and a possible explanation for this could be that selective pressures on other somatic characteristics conflict with selection for high growth rates. This could lead to either a delay in the establishing of equilibrium, or in the case of pleiotropic coupling of the two traits, a stable polymorphic situation within the population. If this were the case, the rate of polypide turnover could well be such a characteristic. Overall fitness of a fast-growing genotype could be reduced by a relatively high polypide turnover rate, which could result in similar overall fecundity to that of a slower-growing genotype which turns over its polypides at a slower rate, thus maximizing the number of functional zooids that are able to reproduce. Also, high polypide turnover could result in the number of non-feeding zooids in the colony being increased, because a higher proportion of time per zooid would be spent regenerating; as a consequence, food uptake should be reduced, with a concomitant decrease in fitness.

With regard to 3) above it must be emphasised that there almost certainly is a metabolic cost involved in both polypide degeneration and regeneration, despite the possibility that some nutrients might be contributed to the colonial

nutrient pool after the breakdown of the polypide (Palumbi & Jackson, 1983), or to the production of the new polypide within the zooid. It appears unlikely that the additional metabolic input from one or several additional polypide generations would be sufficient to compensate for this metabolic expenditure although such data are presently lacking. This particular scenario might result in colonies being selected for higher polypide turnover, as opposed to the former two scenarios.

Rejuvenatory or not, it can be argued that production of more than one polypide generation by the same zooid is a more favourable strategy than the production of only the single polypide which risks premature zooid death (by, for example, predation) and which may lead to only thin bands of active zooids around the colony periphery (Dyrynda, 1981). The fact that there is a colonial nutrient pool, with active transport of metabolites within the colony (Best & Thorpe, 1985), and the expectation that fecundity in bryozoans is directly proportional to the number of functional zooids, both illustrate the benefits of the former strategy and the selective pressure on maximizing the number of functional zooids. Repeated regeneration of polypides may well be the more advanced strategy.

The genotype x food concentration interaction observed for *E. pilosa* was attributable to the different genotypes contrasting in their relative performance (polypide life spans, mean relative growth rates, PAR, RCP) according to food concentration. Because different experimental food concentrations essentially represent spatially different

environments, this result represents further experimental evidence for genotype x environment interaction, observations of which are generally scarce amongst animals (Hughes, 1992). Genotype x environment interaction is a basic underlying assumption of the Tangled Bank hypothesis (Bell, 1982, 1987) pertaining to the evolution of sex. Despite the one anomalous tank (see above), the potential importance of genotype x environment interaction in the present context is perhaps best illustrated by the observation that such interaction was sufficiently intense to reverse the rank order of relative genotype response to treatments for all measured parameters (see Figures 2.3-2.5). It is particularly striking that the relative difference in mean relative growth rate for genotype 2 *versus* genotypes 1 and 3 was comparatively greater at the two lower food concentrations; this suggests the possibility that the relative feeding efficiency of genotype 2 might be best at comparatively low food concentrations. Clearly, a comprehensive understanding of such interactions is a pre-requisite to the analysis of individual performance and fitness under contrasting and variable field conditions.

# CHAPTER3

# GENOTYPIC VARIATION OF GROWTH RATE AND COLONY FORM

## 3.1 INTRODUCTION

### 3.1.1 COLONY GROWTH

In modular organisms, colony growth constitutes a major, if not the most important element of fitness. Size maximization in colonial animals has a direct effect on fecundity (e.g. Hayward & Ryland, 1975; Thorpe, 1979; Wood & Seed, 1992), regenerative capacity, competitive ability and resistance to predators, diseases and catastrophes (e.g. Jackson & Coates, 1986). The likelihood of survival of parts of the colony increases as a function of colony size. In situations of overgrowth competition, colonial animals can have size refuges from competition by exceeding a certain size limit (Sebens, 1982). Apart from the fact that the risk of physical or biological disturbance will increase with substratum area occupied, there are generally no limits to the benefits of indeterminate growth (Jackson, 1979). In bryozoans, colony growth takes place generally as the addition of new zooids by asexual budding (Ryland, 1971); once a zooid has been fully formed, its skeletal growth comes to a standstill and zooid dimensions remain constant (Hughes & Hughes, 1986b).

Growth functions in bryozoans are highly dependent on the mode of growth of the colony; circular encrusting colonies will grow quadratically if they expand at a constant rate (Wass & Vail, 1978). Because of the allometric relationship between colony perimeter and colony area, this can be achieved only by constantly increasing the zooidal budding rate; Hughes & Hughes (1986b) showed that this is the case in *E. pilosa*. *E. pilosa* also compensates for the allometric constraint of the two-dimensional mode of growth by increasing its perimeter-area ratio during astogeny, thus maximizing the number of available budding loci. Thorpe (1979) formulated a deterministic model for bryozoans in general, which postulates exponential growth throughout the whole of the growth process, irrespective of the mode of growth.

Erect colonies apparently maintain higher growth rates since their growth is three-dimensional and apparently free from allometric constraints; the arborescent *Flustra foliacea* for example grows exponentially for a period of at least seven years or possible longer (Stebbing, 1971).

Colony growth rates are influenced by a variety of different factors. Genotype appears to play a major rôle; Keough (1989) showed that even full sibs of *Bugula neritina* vary significantly in their growth rates. Growth performance in *Celleporella hyalina* varies significantly between clones (Hughes & Hughes, 1986a; Hughes, 1989), in some cases by factors of up to 4 (Hughes, 1992). Water flow, presumably tightly linked with food supply, appears to be important, as shown by Cancino & Hughes (1987) for the ascophoran

*Celleporella hyalina*. Flow rates that significantly exceed the conditions usually encountered in the natural habitat of a given species, can, however, be detrimental to growth performance (Okamura, 1992; Eckman & Duggins, 1993).

The presence of neighbours reduces growth in *E. pilosa*, regardless of flow rate (Okamura, 1992), probably as a result of reduced feeding rates. Density-dependent reduction of growth rates has also been reported for *Celleporella hyalina* (Cancino & Hughes, 1987) and *Membranipora membranacea* (Ellison & Harvell, 1989; Harvell *et al.*, 1990). Keough (1986) showed that colonies of *Bugula neritina* growing on the distal ends of seagrass blades attain larger sizes than colonies on the basal ends, presumably as a result of higher flow rates around these parts of the plants.

Colony growth rates may vary significantly both with different types and quantities of diet (Winston, 1976; Jebram & Rummert, 1978; Hunter & Hughes, 1991, 1993a). Limited food supply also leads to reduced growth in *Celleporella hyalina* (Hughes, 1989); but, as with most environmental parameters, there appear to be food concentrations that are optimal for a given species, and food in excess of these will lead to a reduction in growth (Hunter & Hughes, 1993b). This perhaps is attributable to fouling of the colony itself and clogging of the lophophores.

Colonies of *Membranipora membranacea*, *Conopeum reticulum* and *E. pilosa* grown at different temperatures in the laboratory showed a positive correlation between temperature and colony growth rate (Menon, 1972); similarly, colonies growing under natural conditions in the field

exhibit higher growth rates in the warmer season (Kitamura & Hirayama, 1984; Keough, 1986; Pätzold *et al.*, 1987), this apparent effect might, however, be confounded by the concomitant increase in food concentrations. Salinity, too, has a direct effect on colony growth in bryozoans, again with growth and salinity being positively correlated (Kitamura & Hirayama, 1985).

### 3.1.2 COLONY FORM

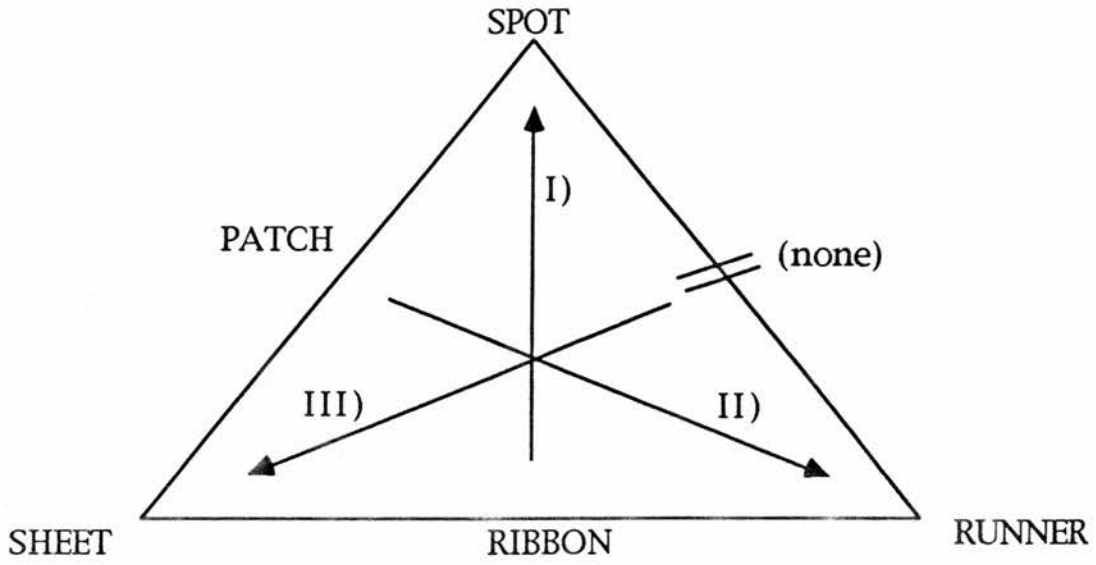
Like colony growth rate, colony form appears to constitute a strongly fitness-related trait; the adaptive significance of colony form and morphology in modular organisms repeatedly has been subject to discussion (e.g. Kaufmann, 1970; Stebbing, 1973a; Buss, 1979; Willis & Ayre, 1985; Hughes & Hughes, 1986b; Jackson & Coates, 1986; Rubin, 1987; Bishop, 1989; Okamura, 1992). The first extensive model of the adaptive significance of colony form was developed by Buss (1979); its basic underlying assumption is that colony morphology in encrusting colonial animals represents an adaptation either for competition for space with other encrusting organisms or for the exploitation of spatial refuges. Spatial refuges are positions on a given substratum that constitute some means of escape from mortality processes and hence provide higher relative fitness for an organism than for its conspecifics in other positions on the same substratum. Colonial organisms respond to spatially unpredictable refuges (*i.e.* the position of the refuge changes over time or the refuge was not available at the time of settlement but became

available later) by adopting a runner-like growth form in order to increase the probability of encountering a spatial refuge (Buss, 1979). The probability of locating spatial refuges increases with the distance from the point of settlement; consequently, compact, sheet-like forms would be expected to have lower frequencies of refuge occupation. Data from a general model and experimental studies suggest that sheet-like species are indeed generally better competitors for space and hence less dependent on spatial refuges. Buss also hypothesized that colonies displaying elongate, directional growth ("runners") should have higher growth rates than sheet-like forms; experimental evidence for this is, however, scarce. Directional growth might represent an adaptation for the location and exploitation of spatial refuges that are distributed in an unpredictable fashion. Sheet-like forms are assumed to represent a confrontational strategy, whereas "runners" represent a refuge exploitation strategy. Between the endpoints of the model (sheets/runners) lies a continuum of intermediate morphologies.

Bishop (1989) criticised Buss' (1979) model for assuming that uniserial growth is both the source of competitive inferiority and the solution to the problem; he argued that traits that are advantageous in a productive, prime microhabitat, might be disadavantageous in peripheral areas of reduced productivity (= spatial refuges). For example, many multiserially growing species overgrow other bryozoan colonies, epibiotically rather than competitively, which is impossible for multiserial species. The ability of runners to colonise grooves, maze-like or strongly three-dimensional

structures is exclusive to that group and hence a positive adaptation to these environments rather than a fugitive strategy. Bishop also extended Buss' model by adding the concept of the "spot" colony, a compact, circular or subcircular colony of smallish size (e.g. *Celleporella hyalina*); Bishop claimed that spot colonies are best adapted for the exploitation of spatial refuges because compact growth is critical when spatial refuges are often small and spatially restricted.

Fig. 3.1: Classificatory model for two-dimensional encrusting growth in bryozoans. Arrows within the triangle characterize the relationship between an endpoint category and the remaining two endpoints. Arrow (I): increased exploitation of spatially predictable refuges; restricted/determinate growth. (II): increased exploitation of spatially unpredictable refuges; directional growth. (III): increased independence from colony-scale refuges; pre-emption and defence of substratum space; expansive, equi-dimensional growth.




The revised model (Fig. 3.1) is triangular, with spot, sheet and runner colonies as endpoints and continuous variation between the endpoint extremes (this excludes the spot-runner axis, as there are no conceivable intermediates for this). In Bishop's terminology, intermediates between sheets

and runners are termed "ribbon", a description which fits *E. pilosa* rather well; intermediates between spots and sheets are referred to as "patch" colonies.

Rubin (1987) carried out an extensive computer simulation involving refuge location by colonies of different shapes and of different sizes; his results suggest that colony form is almost of negligible importance in refuge location, whilst colony size and refuge density play a much more critical rôle. Rubin argued that species such as *E. pilosa* maintain high growth rates because they are poor space competitors and need to exploit spatial refuges; in turn, an elongate growth form like that of *E. pilosa* is a prerequisite for maintaining a high growth rate.

Three-dimensionally growing colonies have, to date, not been included in growth form models, but there is some evidence to suggest that arborescent species do also exploit spatial refuges; Walters & Wethey (1991) found that larvae of the arborescent bryozoan *Bugula neritina* and the pedunculate ascidian *Distaplia occidentalis* settle in crevices or at the bases of bumps, where their early colony stages are, for example, least susceptible to the effects of fish predation. Protection is also afforded from mechanical damage due to abrasion. In contrast, they found the larvae of the encrusting *Membranipora membranacea* to settle preferentially on the highest available locations of complex surfaces; this may provide the early colony stages with a refuge from overgrowth by space competitors.

Silén (1987) investigated the growth pattern of *E. pilosa* and argued that it is more primitive than that of most

other cheilostomes, as indicated by the occurrence of uniserial growth, the existence of a proximal budding locus, and the lack of distal broadening of zooid rows. Colony growth in *E. pilosa* is "sectorial", in that the main growth axes alternate with areas of distolateral budding. Differences in zooid growth rates can alter the width of the main growth axes; perturbations of the growth pattern result from the collision of rows that have originated distolaterally from zooid rows of the main growth axes. Repression of the distolateral loci leads to uniserial growth; this may occur under conditions of food scarcity, but also under circumstances of severe spatial limitation (e.g. when overgrowing the much larger, spiny ctenostome bryozoan *Flustrellidra hispida* (Stebbing, 1973a)).

Okamura (1992) found neither water flow nor conspecific neighbours to have a significant effect on colony form in *E. pilosa*; she concluded from this that the predictions of the adaptive growth morphology models do not apply to this species because colony morphology was not affected by unfavourable environmental conditions. Rather, it was suggested that the stellate growth form of *E. pilosa* is produced under conditions of unrestricted growth on an unobstructed flat substratum. Ryland and Hayward (1977) also claimed that substratum type and texture may influence colony form in this species.

There is evidence for widespread phenotypic plasticity of growth form in many species of colonial invertebrates; for example, Harmelin (1974) found the variation in colony morphology within four mediterranean cyclostome species to be considerable, and related this to the

physical and biological differences (light, water flow regimes, levels of sedimentation, potential mechanical support by e.g. algae or sponges, and space availability) in the habitats occupied. Colony form in these species varied primarily with respect to the extent of ramification in arborescent forms, but in extreme cases even between wholly encrusting and wholly erect morphs. Similarly, clones of the scleractinian coral *Pavona cactus* differ considerably in their growth form *in situ*; clonal identity could reliably be established by electrophoresis and fusion experiments (Willis & Ayre, 1985).

As for colony growth rates, diet appears to be a major determinate of growth form; both qualitative and quantitative diffferences in diets can have major effects on colony form (Winston, 1976; Jebram, 1980; Jebram & Rummert, 1978; Hunter & Hughes, 1993a,b). The general pattern appears to be one of colony compactness decreasing with decreasing food quality and/or quantity. Consistent with this are the findings of Cancino & Hughes (1988) for *Celleporella hyalina*; colonies recruited in spring/summer grow in a somewhat circular fashion, whereas winter recruits exhibit more stellate growth. Seasonal variation can, however, be caused by a variety of factors, including perhaps especially temperature, and it might be problematic to assign the observed effect to any one variable alone.

Whilst phenotypic plasticity of colony form in colonial invertebrates has repeatedly been described, no quantitative investigation of intra- and inter-genotypic variation has been undertaken to date. Only one study has attempted to investigate inter-genotypic variation of colony

form (Hunter & Hughes, 1993b); however, their experimental design was unreplicated at the genotype level, *i.e.* single colonies were assumed to represent different genotypes. Any description of genotypic variation of a trait clearly requires replication of genotypes, because there may be non-environmentally induced variation even between colonies of the same genetic identity.

As alluded to above, modular organisms are ideal for the analysis of genotype effects, because cloning of individual genotypes enables the determination of within-genotype variation, and of broad sense heritability values (Futuyama, 1986). The heritability of a trait is defined as the proportion of the phenotypic variance that is attributable to genetic variation; it is of great importance because it provides a measure of the organism's potential to respond to selective pressure. Heritability in its broadest sense is defined as $h^2_B = V_G/V_P$, where $V_G$ is the variation attributed to genotypic differences, and $V_P$ the phenotypic variation. Broad sense heritability values can be obtained by cloning a number of different genotypes, growing them together under identical conditions, and by dividing the variance of the genotype means of the character in question by the sum of the variance within genotypes and the variance between genotypes. Thus, values range between 0 and 1, with heritability being absolute at 1 and absent at 0. The validity of such a figure will be restricted to the particular population and environment under investigation. By comparison, narrow sense heritability is defined as $h^2_N = V_A/V_P$, where $V_A$ denotes the additive genetic variance which is attributable to additive effects of alleles

within and among loci. The calculation of $h^2_N$, however, requires information on the phenotypes of the parental generation; here, parental phenotypes were unknown, and hence $h^2_N$ values could not be obtained.

The previous experiment described in Chapter 2 did provide an analysis of colony form as well as of growth rate; a criticism of the design, however, is that the colonies from which the experimental clones were derived had been subject to environmental stimuli prior to collection (colonies were of approximately 10-100 zooids, which means that they had been growing in that habitat for at least several days or even weeks). In order to preclude any such environment effects it is necessary to collect specimens prior to their exposure to a specific environment; plastic responses might persist once induced, even after the stimulus itself has been removed.

Replication of genotypes within any one tank in experiment 1 was minimal (n=2); therefore, another requirement here was to observe a greater number of genotypes and also of replicates of genotypes growing in one and the same environment.

Finally, a test of the effect of colony form on colony growth had to be carried out. It has been suggested that *E. pilosa* needs to grow in the typical "ribbon"-like fashion in order to maintain a high growth rate (Rubin, 1987), since the growth potential is a direct function of the number of peripheral budding loci available, which, in turn, increases with the relative colony perimeter. Consequently, a causal

relationship of this nature should be ultimately expressed as a correlation between growth rate and colony form.

## 3.2 MATERIALS AND METHODS

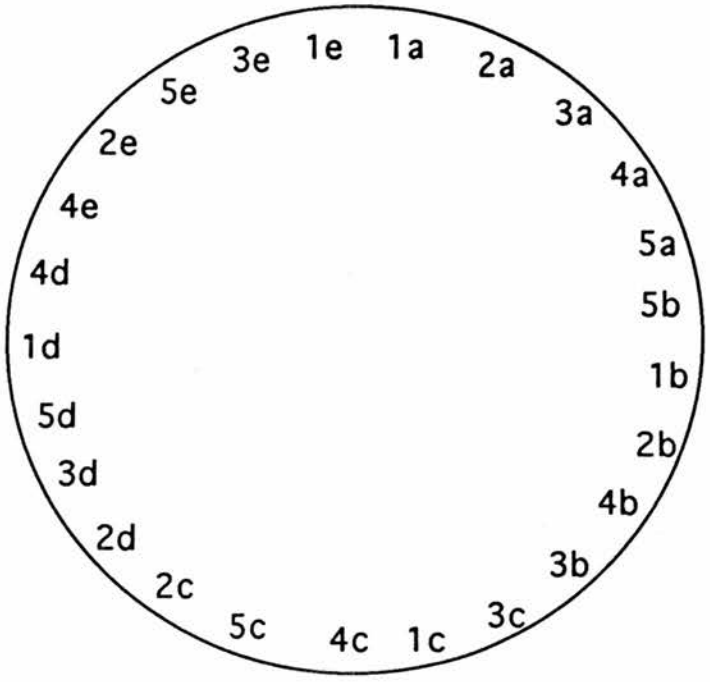
For this experiment, ancestrulae of *E.pilosa* in their very earliest benthic stages were collected on *Fucus serratus* from Kinkell Braes, St. Andrews Bay (see section 1.3). The breakdown of the larval structure in gymnolaemate bryozoans is rapid and immediately follows settlement; this early phase of metamorphosis typically lasts 20 minutes or less (Zimmer & Woollacott, 1977) and is followed by a much longer phase (1 to 6 d) of histogenic differentiation of the ancestrular polypide. The early stage is easily recognizable, as indicated by the absence of a clear organisation of either the larval or ancestrular tissues. Colonies were then replicated employing the method described above (section 1.3). Five genotypes were replicated fivefold each.

Growing colonies together in a single dish requires randomization of the spatial arrangement if positional effects are to be avoided. Although a wealth of randomized block designs exists, circular designs do not seem to be standard methodology and are hence unavailable (R.M. Cormack, pers. comm.). Therefore, a randomized arrangement had to be designed. Randomization of spatial arrangements is necessary not only because of potential differences at the microenvironmental level, but also because of neighbour interactions. Microenvironmental differences here could be

assumed to be negligible, given the artificial and tightly controlled experimental environment. Instead, the focus was on neighbour-balancing of the design, the objective being that the 10 neighbouring positions of the colonies of a genotype were equally divided between all five genotypes, with every genotype neighbouring itself and each of the other genotypes twice (Fig. 3.2). This was done by arranging the colonies in blocks which were assigned letters (a to e), to specify individual replicates within genotypes. Each block consisted of five colonies (including one colony of each genotype). The first block consisted of a simple ascending sequence of numbers 1 to 5. Genotypes then were allocated randomly to the remaining first positions of each of the blocks using random number tables. The last position of the respective preceding block was filled with colonies of the same genotype respectively, thus providing five pairs of colonies of the same genotype and leaving three positions unoccupied between each pair. These were then filled proceeding in a clockwise fashion. Neighbours for each genotype were allocated in ascending sequence of their genotype numbers, using new combinations first before using previously employed combinations for a second time.

Colonies were maintained in a circular pneumatic glass trough of 30 cm diameter and a volume of 7 l. Glass slides were held vertically in a circular perspex rack placed on the bottom of the tank, with all slides oriented vertically with respect to the tank bottom. All colonies faced the same way and therefore were opposed to the unoccupied glass surface of the adjacent slide

Fig. 3.2: Randomized, neighbour-balanced arrangement of colonies in circular tank. Numerals denote genotype numbers, letters denote replicates within genotypes.




A food concentration of 42000 cells · $ml^{-1}$ was maintained throughout the experimental period. Culture conditions were as described in section 1.3. The experiment was conducted over a period of 43 d.

Prior to the definitive experiment, colony growth of the five unreplicated genotypes was measured by establishing total zooid numbers per colony. During the experiment, all other parameters were measured as described above; perimeter-area ratios were not, however, calculated because relative colony perimeter had been confirmed to provide the better measure of colony form because it is free of size bias.

Prior to analysis, all data were tested for normality using the n-scores procedure implemented in MINITAB (Minitab Software, Version 8.2, 1991). Final colony area, final colony RCP and mean relative growth rate then were analysed

by Analysis of Variance, followed by Tukey's Pairwise Comparisons Test. As described above, colony form is best analysed by establishing the relationship between colony area and colony perimeter over the whole range of measurements taken, rather than using only a final measurement. This was obtained both by plots of ln (colony perimeter) against ln (colony area) and fitting least squares regression lines; in addition, an Analysis of Covariance (ANCOVA) was undertaken, using ln (colony perimeter) as response, ln (colony area) as covariate, and genotype as the explanatory variable.

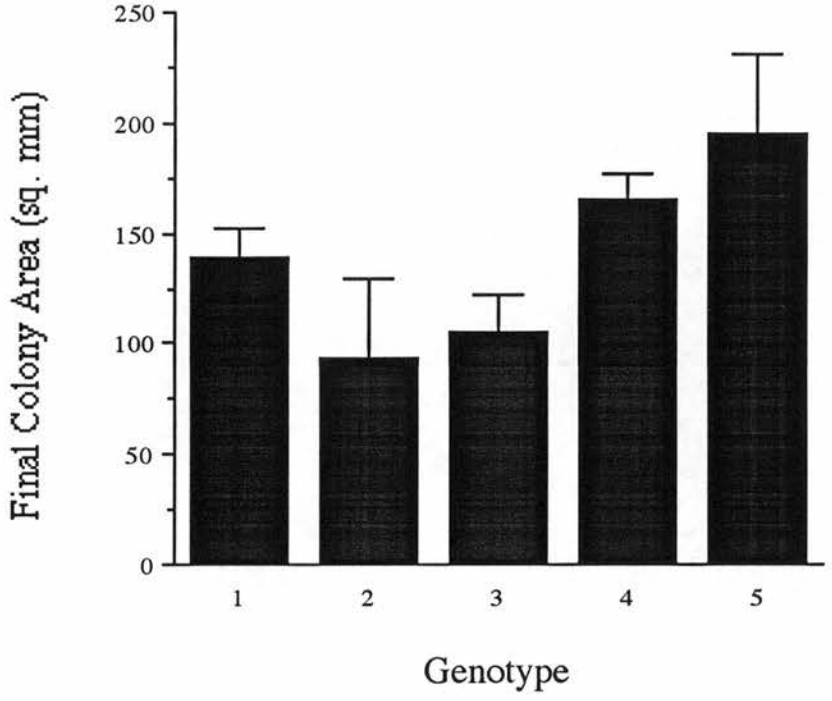
Both growth rate and colony form vary with time; therefore, for the analysis of the relationship between colony form and growth rate, a single measure had to be used for either parameter in order to make analysis possible. Mean relative growth rate, calculated over the whole of the growth period, provides a single measure of the overall growth performance of the colonies and smooths temporal variation. For colony form, coefficients of the regression of ln (colony perimeter) on ln (colony area) were used, rather than RCP, because they provide an overall measure of colony shape which is independent of time. A high value for the regression coefficient indicates a stellate colony form, with low values for a more lobate one. Because individual observations pertain to individual colonies rather than to genotypes, and because genotypes are expected to differ with respect to both parameters, a comparison of the relationship between the parameters had to be carried out at the genotype level. ANCOVA was here used, with R as the response, regression

coefficients as the covariate, and genotype as the explanatory variable.

## 3.3 RESULTS

ANOVA of final colony area and mean relative growth rate showed highly significant genotype effects for both parameters (Table 3.1; Figs. 3.3, 3.4). Final colony area and mean relative growth rate showed essentially the same trends, but within-genotype variation was more pronounced for R than for final area, as indicated by the lower *F*-ratio. This was a consequence of R taking into account both initial and final colony sizes. A comparison of growth performance rank orders between the pre-experimental period, in which the growth of the unreplicated colonies was measured, and the experimental period showed that there are no differences when R is employed as the growth measure (Gt.5 > Gt.1 > Gt.4 > Gt.3 > Gt.2; see also Figs. 3.3 and 3.5 and Tables 3.1 and 3.2); the use of final area, however, results in a reversal of rank orders between genotypes 1 and 4 (Gt.5 > Gt.4 > Gt.1 > Gt.3 > Gt.2). This is intuitive because the calculation of R smooths temporal variation in growth performance, and different genotypes did indeed appear to have different temporal growth patterns (Fig.3.6). That could account for the reversed rank order of genotypes by final colony area. For example, colony growth increased in four out of five replicates of genotype 3 from week 1 to week 2, whereas all colonies of genotype 1 showed a

Fig. 3.3: Two different measures of colony growth performance. Top: colony area at the end of the experiment, expressed as means of the 5 colonies of each genotype. Bottom: mean relative growth rate over the whole of the experimental period, again as means of all colonies of a genotype.


Final Colony Area (sq. mm)
250
200
150
100
50
0
1
2
3
4
5
Genotype

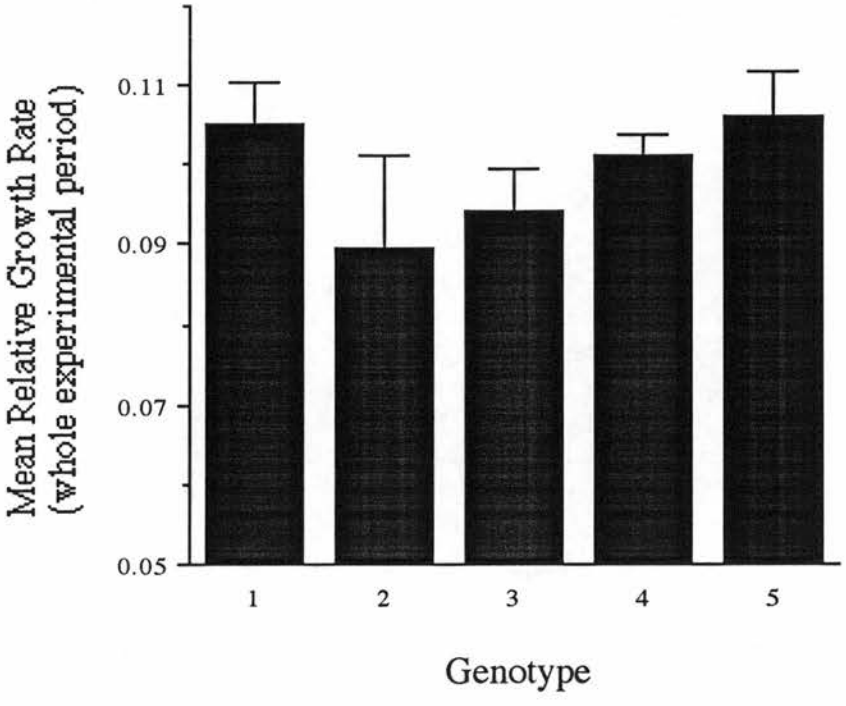

Mean Relative Growth Rate
(whole experimental period)
0.11
0.09
0.07
0.05
1
2
3
4
5
Genotype

Fig. 3.4: The colonies at the end of the experiment, after a growth period of 43 days. Replicates of individual genotypes are grouped in columns, genotypes are arranged no.1 to no.5 from left to right.

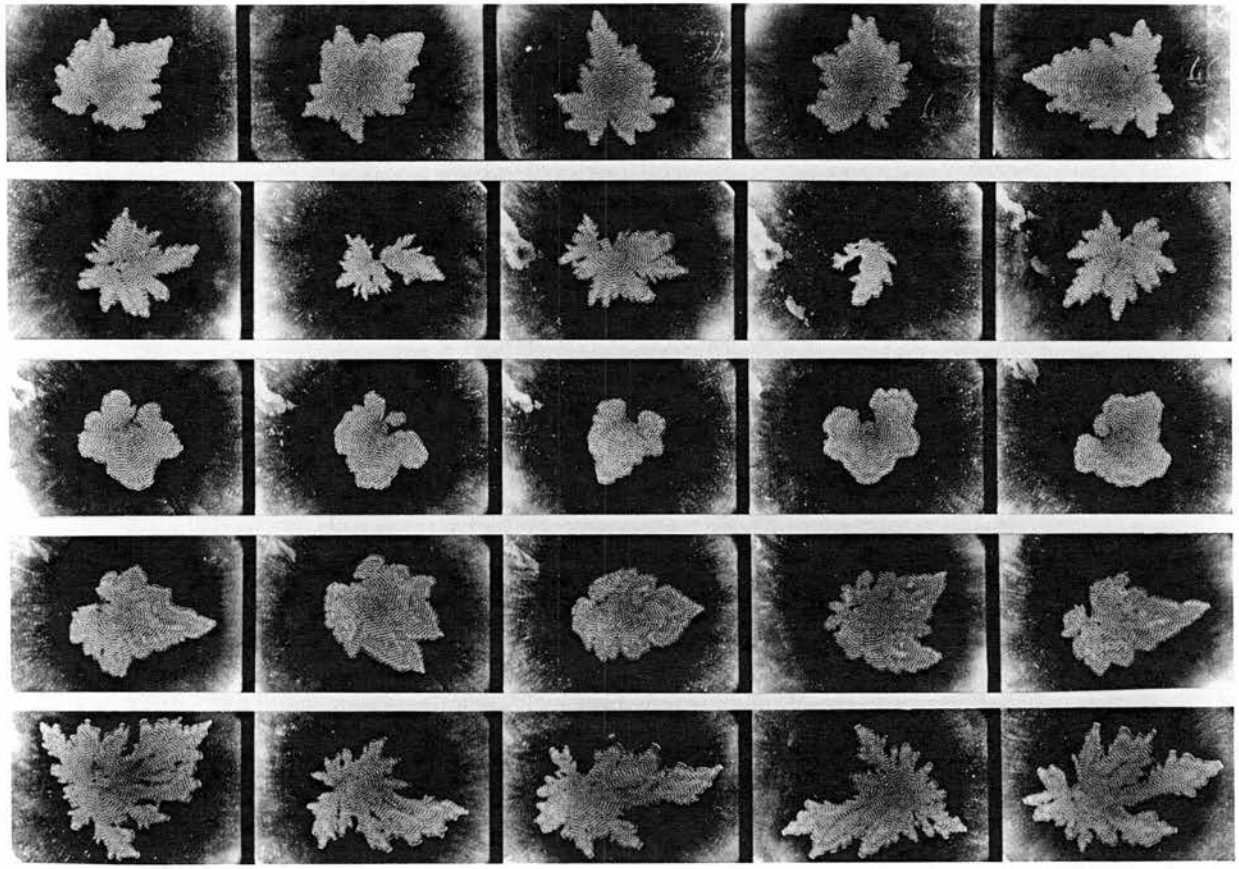
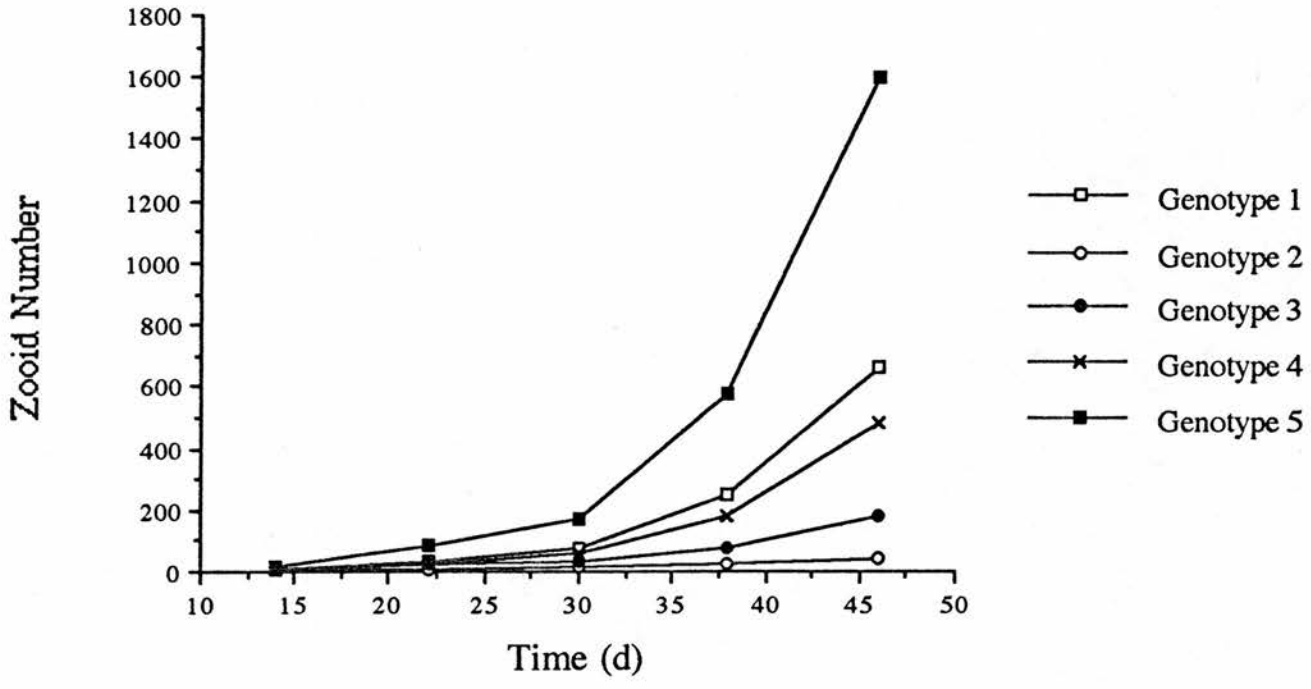


Fig. 3.5: Growth performance of the five unreplicated genotypes prior to the experiment proper. Note that the parameter used here is total autozooid number rather than colony area.


Zooid Number
1800
1600
1400
1200
1000
800
600
400
200
0
10
15
20
25
30
35
40
45
50
Time (d)
Genotype 1
Genotype 2
Genotype 3
Genotype 4
Genotype 5

decrease in growth rate at the same time. Despite these differences, an overall trend is recognizable for most colonies, of R declining after an initial increase and then apparently levelling out or increasing slightly towards the end of the experiment.

Exponential colony growth has been reported both for bryzoans (Bushnell, 1966; Hayward & Ryland, 1975; Thorpe, 1979) and colonial ascidians (Yamaguchi, 1975; Grosberg, 1982; Boyd et al., 1986). In the present study, however, growth was not exponential throughout the whole of the experimental period (Fig. 3.7). The temporal growth pattern becomes clearer when ln (colony area) is used, rather than colony area (Fig.3.8): initially, growth is exponential, as indicated by the relative straightness of the first half of the growth trajectory (Causton, 1983), and then slows down almost simultaneously in all of the genotypes, albeit at differing colony sizes. This is indicative of logistic or Gompertzian colony growth; consequently, both types of curves were fitted to the $\log_{10}$ transformed data. Residuals of both curves indicate that the logistic function (equation 3.1) in most cases provided a better fit than did the Gompertz function (equation 3.2), and was confirmed by a paired t-test (mean difference 0.040, P=.0086). This is not surprising, because the Gompertz function, although quite similar to the logistic function, was designed originally in connexion with actuarial studies, rather than as an analytical tool for vegetative growth processes. The Gompertz function is now typically used to model growth of, for example, vertebrate organs (Causton, 1983). The two

Fig. 3.6: Mean relative growth rates for individual colonies, calculated for weekly intervals, and plotted by genotype.

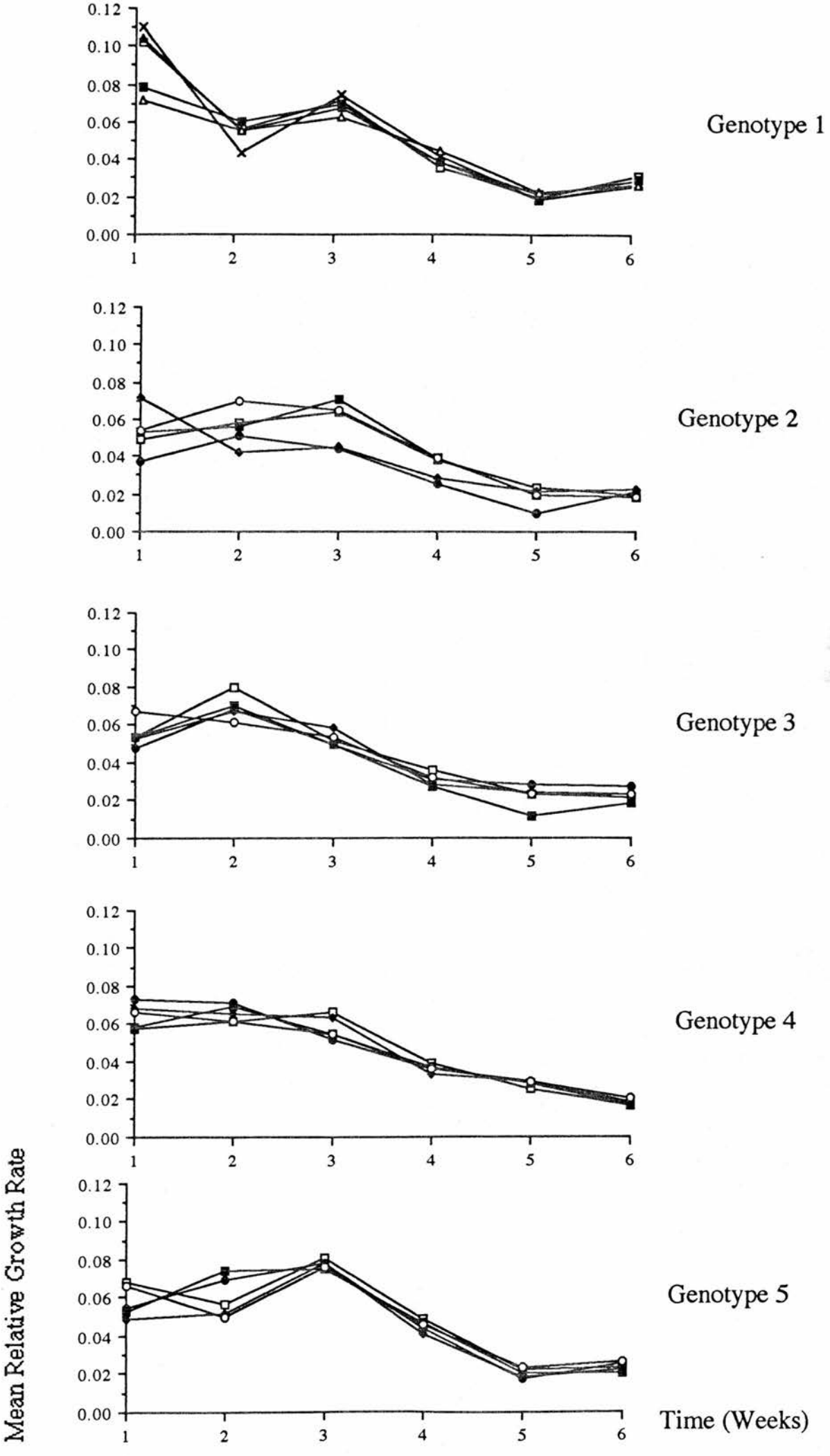

Genotype 1
Genotype 2
Genotype 3
Genotype 4
Genotype 5
Mean Relative Growth Rate
Time (Weeks)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
1
2
3
4
5
6

Fig. 3.7: Colony growth by area, over the whole of the experimental period, plotted by genotype.

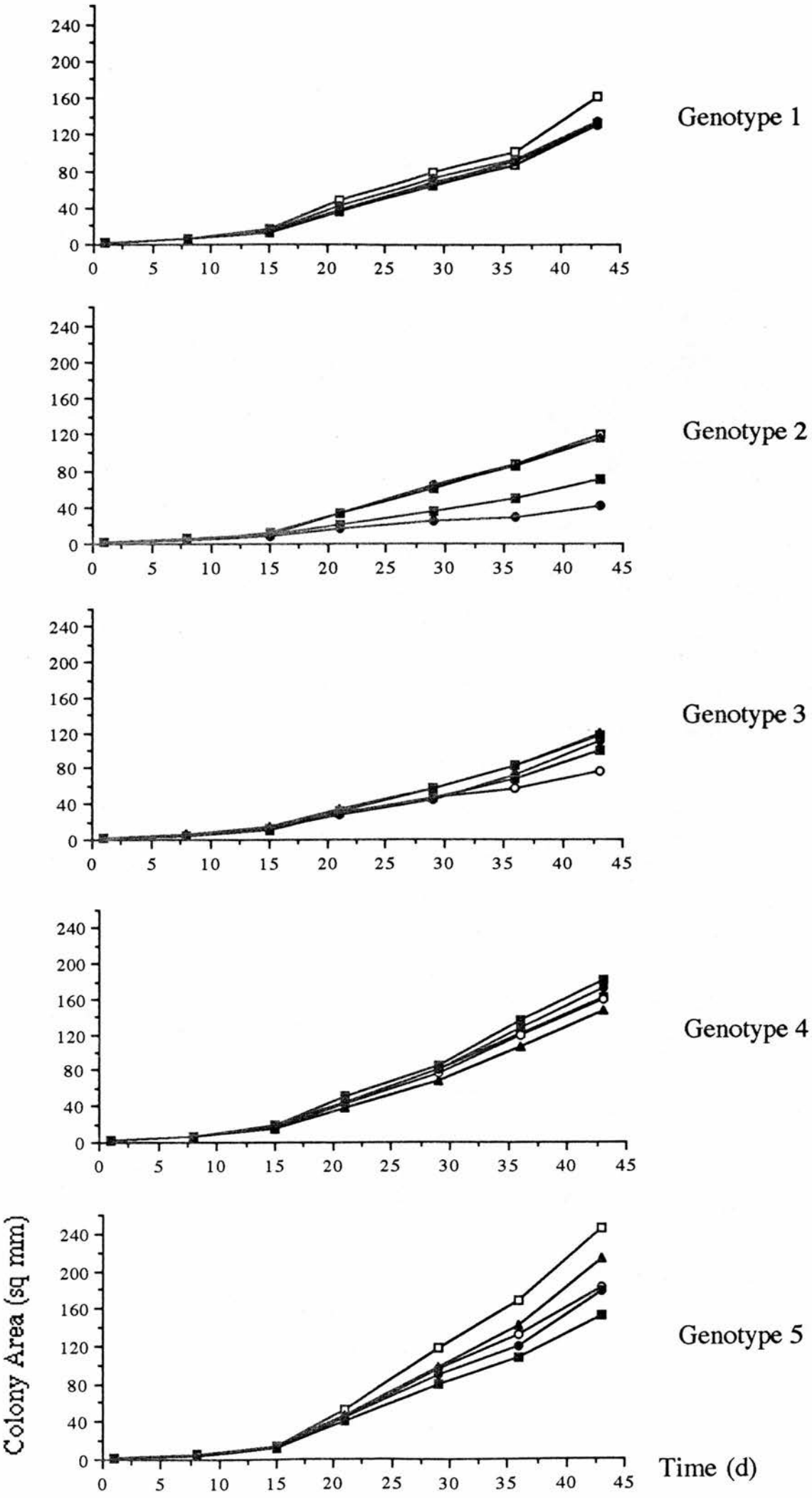

Genotype 1
Genotype 2
Genotype 3
Genotype 4
Genotype 5
Colony Area (sq mm)
Time (d)
0
40
80
120
160
200
240
0
5
10
15
20
25
30
35
40
45

Fig. 3.8: Colony growth by area, on logarithmic scale.

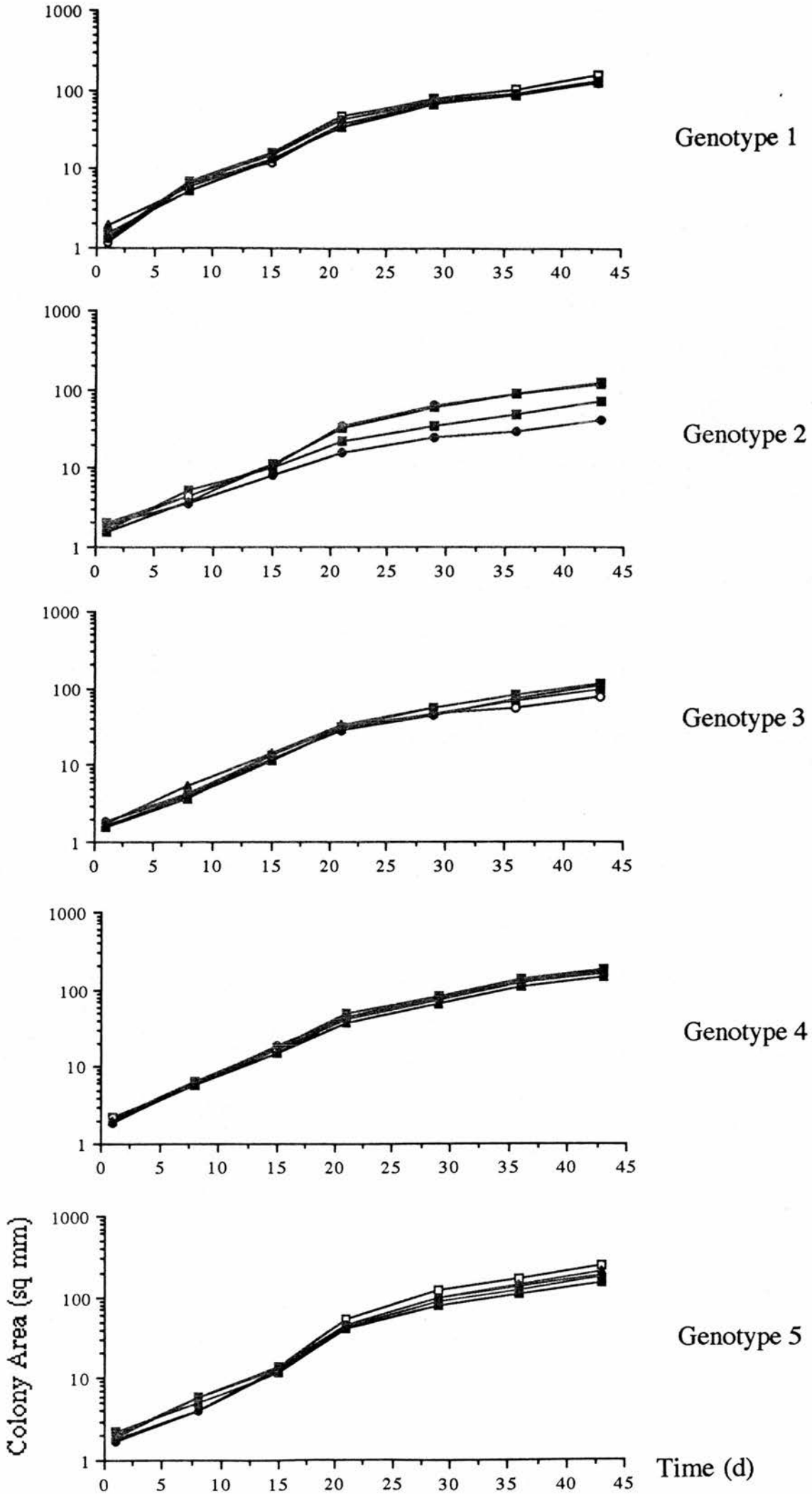

Genotype 1
Genotype 2
Genotype 3
Genotype 4
Genotype 5
Colony Area (sq mm)
Time (d)
1000
100
10
1
0
5
10
15
20
25
30
35
40
45

Table 3.1: ANOVA and genotype means of mean relative growth rate. Data analysed were colony growth rates from 25 colonies (five genotypes, replicated fivefold each), calculated for the whole of the experimental period (43 d).

| SOURCE | df | SS | MS | *F* | P |
|---|---|---|---|---|---|
| Genotype | 4 | .0010530 | .0002633 | 5.89 | 0.003 |
| Error | 20 | .0008939 | .0000447 | | |
| Total | 24 | .0019469 | | | |

| GENOTYPE | MEAN | SD |
|---|---|---|
| 1 | .10575 | .00519 |
| 2 | .09001 | .01154 |
| 3 | .09471 | .00505 |
| 4 | .10156 | .00243 |
| 5 | .10689 | .00565 |

Tukey's Pairwise Comparisons Test of mean relative growth rate of the five genotypes (an x denotes a significant difference between genotypes):

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | x | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | x | | |

Table 3.2: ANOVA and genotype means of colony area at the end of the experimental period (43 d). Data analysed were colony areas in $mm^2$ from 25 colonies (five genotypes, replicated fivefold each).

| SOURCE | df | SS | MS | *F* | P |
|---|---|---|---|---|---|
| Genotype | 4 | 35067 | 8767 | 13.58 | <0.001 |
| Error | 20 | 12915 | 646 | | |
| Total | 24 | 47982 | | | |

| GENOTYPE | MEAN | SD |
|---|---|---|
| 1 | 138.79 | 12.72 |
| 2 | 93.61 | 36.12 |
| 3 | 104.80 | 17.13 |
| 4 | 164.50 | 13.07 |
| 5 | 194.98 | 36.03 |

Tukey's Pairwise Comparisons Test of final colony area of the five genotypes (an x denotes a significant difference between genotypes):

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | | | | |
| 3 | | | | |
| 4 | | x | x | |
| 5 | x | x | x | |

functions differ mainly in the assumed values of the asymptotes, which are lower in the logistic function. Thus,

$$(3.1) \qquad \ln y = \ln a - \ln (1 + b\, e^{-kt})$$

$$(3.2) \qquad \ln y = \ln a - b\, e^{-kt}$$

where a is the value of the asymptote, k the y-axis intercept, and $b = e^c$, with c being the constant of integration.

On the assumption that a greater number of parameters in a function generally improves the fit of a model, cubic functions ($y = a + bx + cx^2 + dx^3$) also were fitted to the data. In terms of residuals, these did indeed provide the best fit in most cases, but, coefficients of polynomial functions do not provide any meaningful biological measure (as opposed to the other two models) and those results are therefore not discussed further.

Like the two growth parameters, relative colony perimeter (RCP) was significantly affected by genotype (ANOVA, Table 3.3.). Fig.3.9 shows a plot of RCP for all colonies at the end of the experiment. In addition, ANCOVA of ln (colony perimeter) and ln (colony area), testing for genotype effects over the whole of the experimental period, gave the same result (Table 3.4). A plot of the data, with regression lines fitted to each pool of genotype data, revealed that the relationship between area and perimeter was well defined for each of the genotypes, with $r^2$ values ranging from o.980 to 0.994 (Fig. 3.10). Fitted lines clearly diverge between genotypes, and also diverge strongly from a comparative line representing circles of a similar size range. This suggests that the relationship between perimeter and area is linear at least over the duration of the experiment, and that colonies grow

Fig. 3.9: Relative colony perimeter at the end of the experimental period (t=43 d). Columns represent means of the 5 colonies of a genotype; error bars denote1 standard deviation.

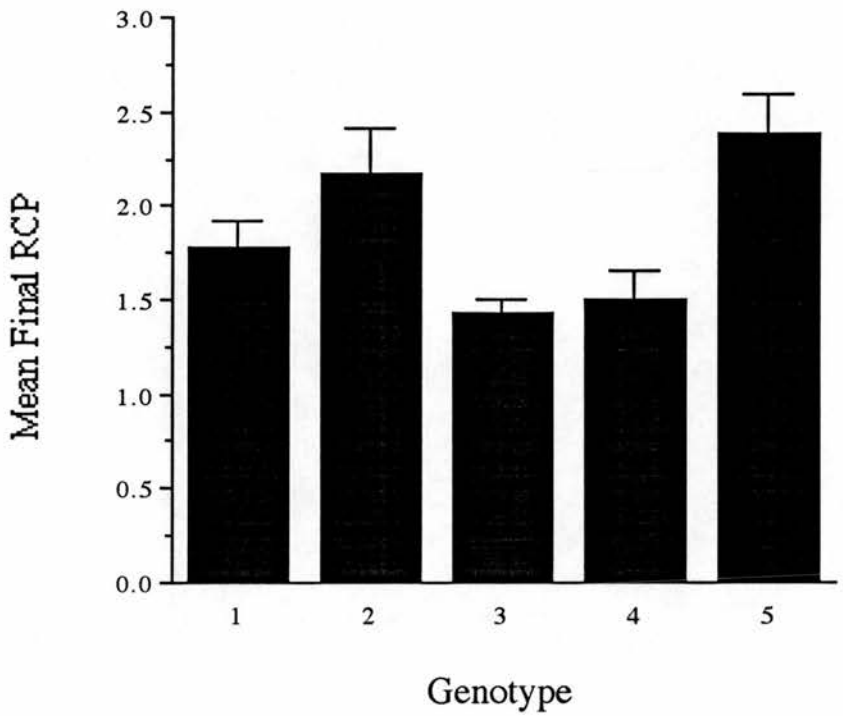

Mean Final RCP
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
Genotype

Table 3.3: Analysis of Variance and genotype means of relative colony perimeter (RCP), calculated as $[\text{perimeter}^2/4\pi\,\text{area}]^{1/2}$, at the end of the experimental period (43 d). Data analysed were from 25 colonies (five genotypes, replicated fivefold each).

| SOURCE | df | SS | MS | *F* | P |
|---|---|---|---|---|---|
| Genotype | 4 | 3.3905 | 0.8476 | 29.24 | < 0.001 |
| Error | 20 | 0.5798 | 0.0290 | | |
| Total | 24 | 3.9703 | | | |

| GENOTYPE | MEAN | SD |
|---|---|---|
| 1 | 1.7864 | 0.1394 |
| 2 | 2.1734 | 0.2373 |
| 3 | 1.4353 | 0.0646 |
| 4 | 1.5107 | 0.1453 |
| 5 | 2.3816 | 0.2096 |

Tukey's Pairwise Comparisons Test of relative colony perimeter of the five genotypes at the end of the experimental period ( an x denotes a significant difference between genotypes):

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | x | | | |
| 3 | x | x | | |
| 4 | | x | | |
| 5 | x | | x | x |

Fig. 3.10: Plot of ln-transformed colony perimeter versus ln colony area. Fitted regression lines represent individual genotypes. For comparison, data of circles of sizes comparable to those of the colonies have been included (open squares). $r^2$ values for individual genotypes: Gt.1 = 0.993; Gt.2 = 0.980; Gt.3 = 0.994; Gt.4 = 0.993; Gt. 5 = 0.989.

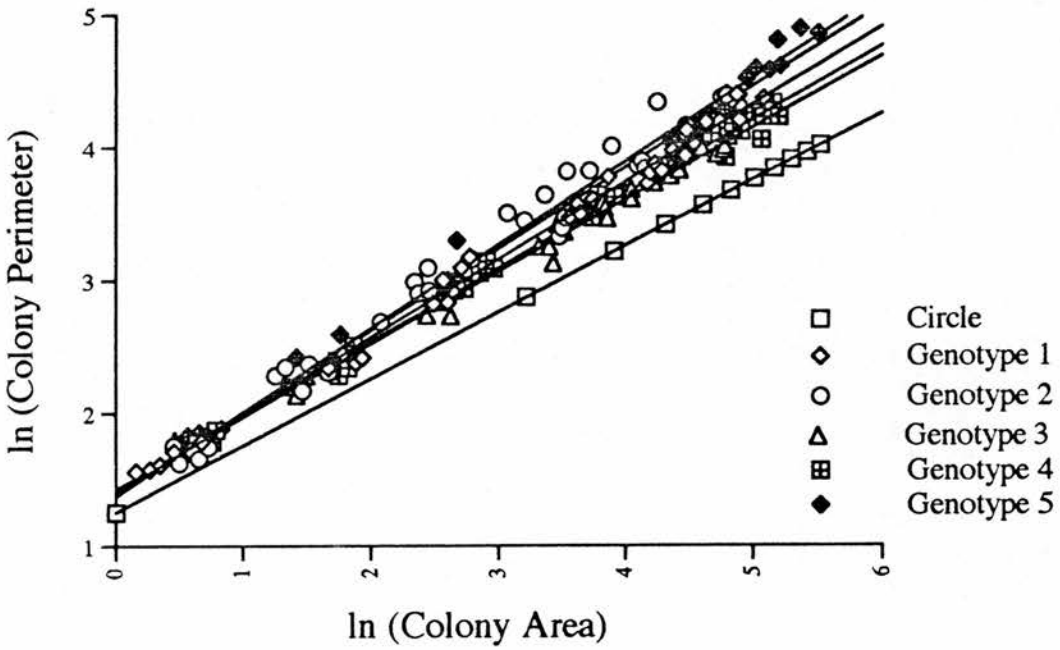

ln (Colony Perimeter)
ln (Colony Area)
1
2
3
4
5
0
1
2
3
4
5
6
Circle
Genotype 1
Genotype 2
Genotype 3
Genotype 4
Genotype 5

Fig. 3.11: Relative colony perimeter of individual colonies over the whole duration of the experiment, plotted by genotypes.

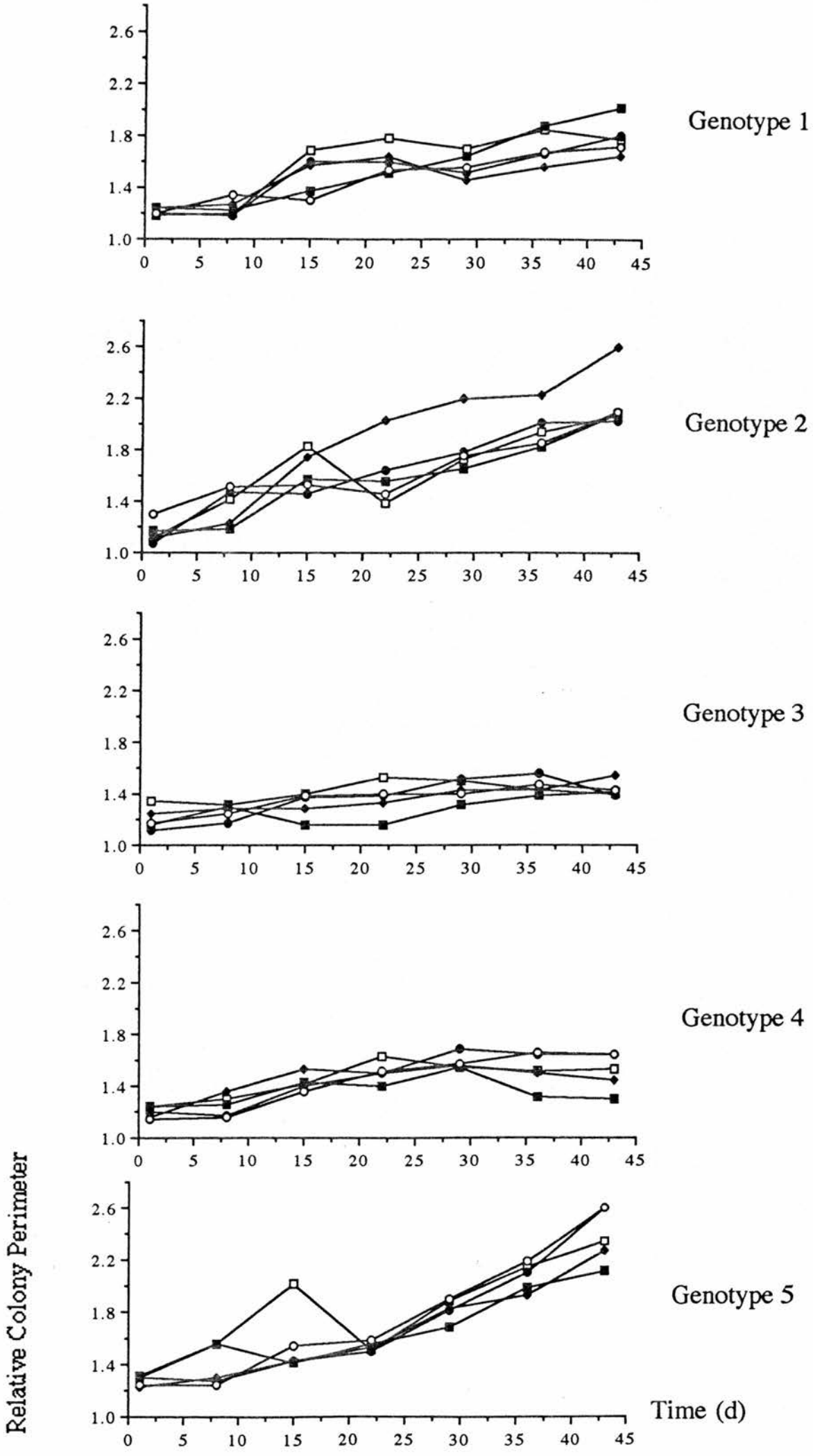

Genotype 1
Genotype 2
Genotype 3
Genotype 4
Genotype 5
Relative Colony Perimeter
Time (d)
2.6
2.2
1.8
1.4
1.0
0
5
10
15
20
25
30
35
40
45

Table 3.4: ANCOVA of colony form. Model was defined as "ln perimeter = genotype, covariate ln area". Data analysed were area and perimeter measurements from 25 colonies (five genotypes, replicated fivefold each), taken in weekly intervals over a period of seven weeks.

| SOURCE | DF | ADJ. SS | MS | *F* | P |
|---|---|---|---|---|---|
| Covariate (ln area) | 1 | 134.186 | 134.186 | 5869.32 | < 0.001 |
| Genotype | 4 | 1.229 | 0.307 | 13.44 | < 0.001 |
| Error | 169 | 3.864 | 0.023 | | |
| Total | 174 | 140.990 | | | |

Table 3.5: ANCOVA of effect of colony form on growth rate. The model was defined as "mean relative growth rate = genotype, covariate colony form regression coefficient". Data were from 25 colonies (five genotypes, replicated fivefold each). Mean relative growth rates were calculated for separate colonies for the whole of the experimental period (43 d). Colony form regression coefficients had been obtained by least squares regression of ln (colony perimeter) on ln (colony area) for each of the 25 colonies (cf Fig. 3.10).

| SOURCE | df | ADJ. SS | MS | *F* | P |
|---|---|---|---|---|---|
| Covariate (colony form) | 1 | .00013209 | .00013209 | 3.32 | 0.084 |
| Genotype | 4 | .00101938 | .00025484 | 6.40 | 0.002 |
| Error | 19 | .00075630 | .00003981 | | |
| Total | 24 | .00191904 | | | |

more stellate as they become bigger. The fact that fitted lines diverge indicates that increases in "stellateness" occur at a genotype-specific constant rate and are independent of colony size. This is supported by a plot of RCP against time (Fig.3.11), which shows that RCP does indeed increase over time for all of the genotypes.

There was no significant effect of colony form on colony growth rate. Regressions of mean relative growth rate on colony form slope coefficients were not significant, and ANCOVA of R with colony form slope as covariate (Table3.5) was non-significant for the covariate ($P = 0.084$).

Heritability values were calculated as $h^2_B$ = MS genotype/(MS genotype + MS error), and were 0.854 for mean relative growth rate and 0.875 for colony form respectively; slopes of regression lines of ln (perimeter) on ln (area) were used for colony form. This is indicative of a comparatively strong element of heritability in both parameters.

## 3.4 DISCUSSION

The temporal pattern of colony growth observed in this experiment was approximately logistic, with growth being exponential initially, and then levelling off asymptotically. Other authors have described similar patterns for bryozoans growing in the field (Bushnell, 1966; Hayward & Ryland, 1975). However, it is obvious that the growth pattern observed here was due to different factors; asymptotic slowing down of

colony growth in those field studies was due to overcrowding in one case (Hayward & Ryland, 1975), leading to substratum space preemption and probably increased competition for food. In another case, decreasing water temperatures in autumn and subsequent large scale polypide degeneration were assumed to be the cause for the observed reduction in colony growth (Bushnell, 1966). In the tightly controlled conditions of the present laboratory experiment, external causes for the slowing down of colony growth can virtually be ruled out.

There are, however, numerous other factors that could possibly account for the observed pattern; foremost, colony form can be assumed to be of major importance. Hughes & Hughes (1986b) have shown that specific colony growth rate in *E.pilosa* declines with increasing colony size, an expression of the constraint imposed on colony growth by the two-dimensional colony morphology. However, their results suggest that *E. pilosa* compensates for this, at least partly, by increasing its relative colony perimeter, and hence the number of available budding loci. Also, the budding rate of peripheral zooids increases as the colony grows bigger, possibly as a result of nutrient translocation, as demonstrated for the cheilostome *Membranipora membranacea* (Best & Thorpe, 1985). It appears both from the present study and from that by Hughes & Hughes (1986b) that this compensation in *E. pilosa* is insufficient to support exponential colony growth throughout a colony's life span. Stoner (1989) also reported a decrease of colony growth rate with increasing colony size in the colonial ascidian *Diplosoma similis*, and assumed similar causes.

Another internal factor with a putative effect on colony growth was mentioned by Bushnell (1966) in his discussion of temporal growth patterns in the freshwater bryozoan *Plumatella repens*:. Terminal polypide degeneration in central, ontogenetically older zooids exceeds the addition of new zooids at some point in the astogenetic development of this species, leading to a continuously decreasing proportion of active zooids in the colony, and thus to reduced nutritional input into the colonial nutrient pool. The life history of *E. pilosa* has only been described in part, and longevity data both for zooids and whole colonies are unavailable. It is therefore unclear how zooid death manifests itself in this species. Gordon (1977) described zooid death in the cheilostome *Cryptosula pallasiana*: following the death of the last polypide, and the subsequent brown body formation, all tissues disintegrate, the operculum falls off, and scavengers enter the empty cystid. By this definition, it can be said that zooid death as such did not occur in the present study. However, in the experiment described in section 2, some zooids entered what is probably best described as an extended non-active phase ("resting") after polypide regression. Some of these subsequently formed new polypides, whereas others remained in that state until the end of the experiment. As already pointed out in section 2.2, this might be indicative of impending zooid death; irrespective of the cause or the duration of inactivity, an inactive zooid will not be able to make any contribution to the colonial nutrient pool. It appears intuitive that if an increasing proportion of zooids in a colony become inactive, relative colony growth rate will decrease. In

a further experiment not included here, polypide life spans appeared to decrease over 11 cycles, whereas the duration of polypide regeneration appeared to increase over the experimental period. Both factors can be assumed to increase the proportion of inactive zooids in a colony, and unless existing active zooids and the addition of new zooids compensate for this in some way, a decrease in colony growth is more than likely. It must be considered in this context that conditions in laboratory experiments such as the present are often highly artificial and hence only rarely allow absolute, rather than relative, observations. If the decrease in relative colony growth rates observed here should indeed have been a consequence of zooid senescence/death, then this might well have been induced, or at least artificially accelerated, by the culture conditions. Both high temperatures and unbalanced diets like the monofood diet used here might have had detrimental effects on the experimental animal.

The genotypic variation of growth rate observed in this study was considerable. Variation of growth rates with regard to genotype is not uncommon; examples are known from other bryozoan species ( Hughes & Hughes, 1986a; Keough, 1989; Hughes, 1989, 1992), but also from plant populations (e.g. Burdon & Harper, 1980) and colonial ascidians (e.g. Stoner, 1989). Via & Lande (1985) have pointed out that major components of fitness can be expected to be under strong directional selection at all times and in different environments. Although countervailing forces might balance this process at some point (Futuyma, 1986), directional

selection will maintain a transient polymorphism until the allele eventually becomes fixed.

Stable, non-transient polymorphisms can be maintained in a variety of ways (Futuyma, 1986; Begon *et al.* 1990):

(1) Fixation of an allele by selection may be prevented by either mutation or gene flow.

(2) Genetic drift can be accountable for a polymorphism because genotypes do not differ significantly in fitness and are hence not subject to natural selection (= flat fitness profiles, see below).

(3) Natural selection may stabilize a polymorphism by acting on different morphs at opposite ends of a gradient, thus creating polymorphic populations of intermediate values.

(4) Under frequency-dependent selection, morphs can be fittest when they are rarest (e.g. rare prey colour morphs that are undetectable by predators), thus forming a stable component of the population.

(5) A polymorphism may be balanced if two different alleles produces homozygotes of reduced fitness, but heterozygotes are fittest because the negative effects of either allele are halved.

(6) A genetic correlation between two traits that are pleiotropically affected by the same locus may maintain variation if one allele codes for one value of the one trait and a different value of the other trait at the same time, whilst other alleles code for other value combinations (Stearns, 1992).

Futuyma (1986) has also pointed out that polymorphisms are frequently found in species which display an active choice of habitat; this is at least partly the case for *E. pilosa*, as suggested by its preferential settlement on the distal parts of *Fucus serratus* thalli (Ryland & Stebbing, 1971). Whether or not a component of gregariousness is involved in its settlement behaviour is to date unresolved, but the distribution of E. pilosa colonies on Fucus serratus departs signficantly from the Poisson distribution, suggesting a non-random pattern situation (Ryland & Sykes, 1972).

Furthermore, polymorphisms are more likely to occur in situations where differentially adapted genotypes compete for different resources; frequency-dependent selection will then act in a stabilizing fashion.

Scenario (1) above can not be dismissed altogether, but seems to be unlikely considering the continuous variation and even spacing of genotype means, which suggests a stable polymorphic situation rather than a chaotic genetic contamination. Scenarios (2)-(5) are clearly inadequate explanations for the observed variation, since the mode of selection acting on growth rate will be directional. (2)-(5) assume other modes of selection). With regard to (6), it must be said that this is a likely and plausible explanation, as already discussed in Chapter 2. Colony growth rates could, for example, be genetically correlated with polypide life spans.

Growth rate can be expected to be a component of major importance to fitness in bryozoans (Thorpe, 1979), because fecundity in most species will be a direct function of zooid number. Also, it is conceivable that egg number plays a

subordinate rôle relative to zooid number since eggs are large and few (10-20 eggs per zooid, about 80 μm in diameter; Marcus, 1926). The range of egg numbers will probably be rather small since a zooid can only accommodate a certain number of eggs. This can be demonstrated on a simple numerical example, based on data from this experiment. At the end of the experimental period, the biggest and the smallest colonies measured 1638 and 286 zooids respectively, a nearly sixfold difference [zooid numbers were calculated from colony areas and mean zooid sizes (which were genotype-specific and obtained by measuring zooid area on 10 zooids from each colony)]. If a median value of 15 eggs per zooid is assumed for the largest colony, the smallest colony would require 86 eggs per zooid to achieve equal fecundity, and this after a period of only 43 d. Although trade-offs between somatic and sexual investment are not uncommon (Stearns, 1992), this is probably far beyond the carrying capacity of a zooid; hence, zooid numbers, as well as age at maturity and length of reproductive period, are possibly more important. Also, the timing of reproduction (semelparity *versus* iteroparity) in colonial invertebrates can differ even within the same species (Grosberg, 1982). Similarly, egg size is not likely to be so important in a species that has planktotrophic larvae; size increase in the cyphonautes larvae over the pelagic period is marked (Ryland, 1976) and will probably depend on parameters such as food availability and temperature, more than egg size.

Rubin (1987) has deduced that *E. pilosa* is a poor space competitor and grows in its typical elongate growth form

in order to maintain a high growth rate which is needed for the exploitation of spatial refuges. This inference is, however, somewhat circular, because it assumes that "ribbon" morphology is both the source of and the solution to one and the same problem. It is probably more adequate to assume that "runner" or "ribbon" morphologies represent positive adaptations to certain microenvironments that are inaccessible for "sheet" colonies, rather than fugitive strategies (Bishop, 1989).

The interpretation of Bishop's model (Fig.3.1), on the basis of the present findings depends largely on fitness values assumed for the different colony forms. If "runner" and "sheet" colonies represent positive adaptations to certain microenvironments, and if the exploitation of these environments is correlated closely with certain trade-offs (e.g. reduced risk of mortality *versus* reduced fecundity due to spatial restrictions, in the case of a "runner" colony growing in a groove), then the trait would be selectively neutral and its fitness profile (Robertson, 1955; Stearns, 1992) could be expected to be relatively flat. If a trait has a flat fitness profile, strong variation around its optimum can often be observed (Stearns, 1992). Microenvironment-specific mortality rates certainly can be expected to be relatively higher for unprotected colonies growing on flat unobstructed surfaces, which are exposed to the effects of both physical and biological disturbance and perturbation (e.g. abrasion, predation, overgrowth).

In most cases, however, spatial refuge exploitation in the narrow sense will not be critical to *E. pilosa*. The

majority of colonies of this species grow on flat unobstructed surfaces such as the thalli of *Fucus serratus* (Ryland & Hayward, 1977; pers. observ.), where spatial refuges including groves or pits are few. In these epibiotic assemblages, overgrowth competition is a major source of mortality (e.g. Stebbing, 1973a,b; Karlson, 1978; O'Connor *et al.*, 1980; Russ, 1982; Rubin, 1985; Gappa, 1989; Miles & Meslow, 1990; Turner & Todd, 1994). *E. pilosa* apparently reduces the risk of overgrowth by other species by settling on the younger parts of the thalli, thus rapidly pre-empting newly formed substratum space (Ryland & Stebbing, 1971). Consequently, the majority of encounters with other colonies are of an intraspecific nature; Stebbing (1973a) has shown that only a negligible fraction of these end in overgrowth in this species, whereas most end in "ties", often with subsequent redirection of colony growth. This places strong emphasis on the pre-emption of substratum space, since overgrowth of other colonies does not appear to be a major factor. In this situation, a stellate colony could be expected to be at an advantage, because it is better able to fill in remaining spaces than can lobate colonies, thus maximizing colony size.

If the present findings can be generalized and extended to other species, colony form could play an important rôle in overgrowth competition. It has been shown that the angle of encounter between bryozoan colonies can have a significant impact on the outcome of the encounter (Rubin, 1982; Turner & Todd, 1994); colony form has, however, not been considered to be of importance. A stellate bryozoan colony (Fig. 3.4) will confront an opponent in an overgrowth

encounter at a variety of encounter angles, since its zooids are not growing in a more or less parallel fashion as for lobate colonies. Overgrowth might then ensue somewhere along the contact area, not necessarily at the point of first contact but rather at a point where the encounter angle between the colonies is favourable. The phase of colony margin elevation over the adjacent colony is certainly critical in the overgrowth process, since the overgrowing colony will be able to grow in an essentially unhindered fashion once zooids have been established on the competing colony (pers. obs.). If intraspecific differences in colony form are generally as marked as those found in this study, and if colony form does indeed affect the outcome of inter- or intraspecific encounters, then some of the "stochastic" intraspecific variation observed in studies on overgrowth competition might be explained. On the basis of the apparently high heritability of colony form, it might be predicted that different genotypes will have different potential for colony overgrowth. A possible limitation to this is, of course, that colony form might change as a response to the encounter itself.

Variation of colony form amongst natural populations also can be a result of genetic contamination from other populations (see above). *E. pilosa* is one of the few bryozoan species with a long-term planktotrophic larval stage (Ryland & Hayward, 1977), and hence has the potential for larval dispersal over significant distances. If there was an influx of larvae from other populations, the observed polymorphism might not be stable in time, because dispersal distance and success might themselves be erratic due to biotic

and abiotic factors (including currents, storms and differing predation levels). However, as for growth rate, the even spacing of genotype means here suggests a stable polymorphism rather than a gene flow effect. This latter hypothesis lends itself to testing by repeating the experiment several times over an extended period. This would result either in significant between-experiment variation (in the case of genetic contamination events), or consistently yield results similar to the present, in the case of a stable polymorphic situation.

However, in the light of the considerations about the effect of colony morphology on growth rate, and also the present findings, it might be inadequate to consider colony form and growth rate to be independent parameters. Here, sample size at the genotype level was probably inadequate to provide a sound basis for investigating the relationship between growth rate and colony form; however, a trend was detectable of colony form affecting growth rate, as indicated by the comparatively high P-value in the ANCOVA of growth and form (Table 3.5). Assuming that two colonies differ in form, but are equal in terms of zooidal budding rate and pattern (relative frequencies of distal vs. lateral budding) a stellate colony can be assumed to achieve faster colony growth, as is indeed the case in the colonial ascidian *Diplosoma similis* (Stoner, 1989). For two-dimensional colonies, the colony growth will in all likelihood be strongly affected by the number of budding loci available, which in turn is a function of colony perimeter. Broad sense heritability values for both traits in this experiment were high, and closely similar. This

could be expected in a situation where there is a functional dependency of one parameter on the other, because functionally correlated traits would not "behave" like traits inherited independently of each other. In fact, if colony form did have an effect on growth rate, which further experimentation will have to show, then the heritability values calculated for growth rate would be incorrect; growth rates could then depend entirely on the form of the colony and not be heritable at all, but their heritability value would be close to that of colony form. In this context, it is also interesting to note that, as Via & Lande (1985) have pointed out, selection of a trait depending on the selection of another trait can lead to the retardation of a selective equilibrium, which could in turn result in the kind of variation of both traits observed here.

Both colony growth rates and colony form in this experiment were significantly different between the genotypes examined; however, the findings have to be evaluated with caution since falsifying hypotheses relating to genetic variation in the laboratory involves a major pitfall. Stearns (1992) has pointed out that if there is genotype x environment interaction for a trait, removing an organism from its natural habitat and growing it in an artificial environment in the laboratory can produce misleading results depending on the type of environment chosen. Genotype x environment interaction occurs when the reaction norms of genotypes differ in slope over a range of different environments (Fig. 3.12). The differences in reaction norms may then lead to a reversal of rank order between genotype performance if environments at opposite endpoints of the reaction norms are chosen for

experimentation; in extreme cases, they may even mask true genetic differences if the environment used should coincide with the point of intersection of reaction norms.

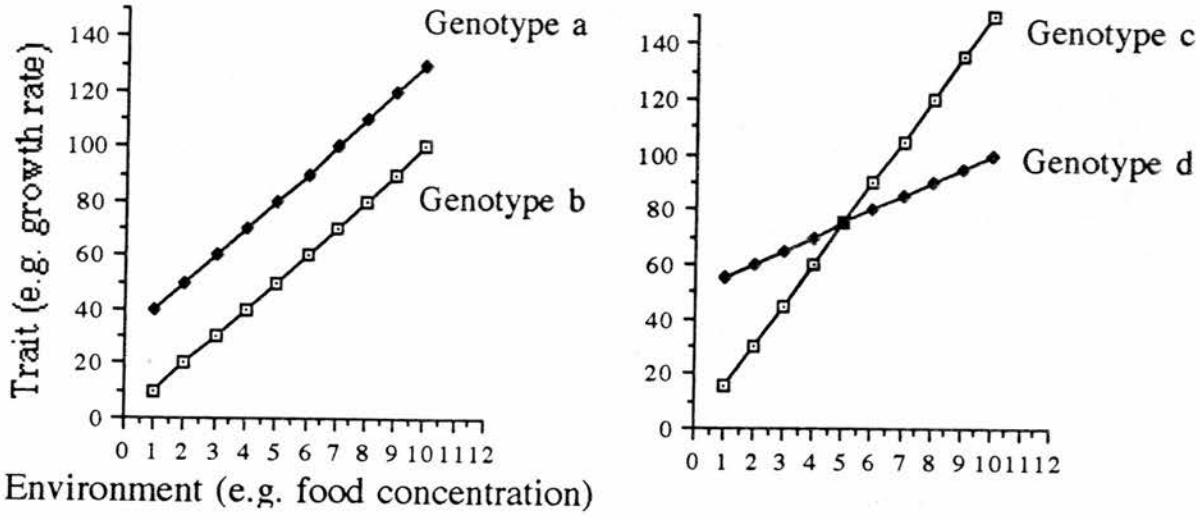



Fig. 3.12: Genotype x environment (G x E) interaction. Slopes of reaction norms in genotypes a and b are identical, hence there is no G x E interaction. Genotypes c and d differ in slope, which implies G x E interaction; here, the reaction norms of genotypes c and d even cross over, an extreme form of G x E interaction.

Genotype x environment interaction also affects heritability values; broad sense heritability for a trait for which there is G x E interaction is defined as $h^2_B = V_G / (V_G + V_E + V_{GxE})$ (Futuyma, 1986). Consequently, if the G x E interaction term changes (by, for example, moving an organism to the laboratory and thus changing its environment), heritability values also will be affected. Obviously, this has implications for the interpretation of the high heritability values observed in this experiment. For the experiment described in Chapter 2, growth rate of colonies in different food concentrations showed significant G x E interaction; colony form was not affected by this if RCP is chosen as parameter, but there was interaction if PAR is used (see section 1.3 for

discussion of the usefulness of either parameter). However, the significant genotype effects for both parameters were strong evidence that the observed between-genotype variation in these experiments is real and does indeed reflect the situation encountered under natural conditions.

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