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An experimental exploration of Waddington's epigenetic landscape

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SUMMARY

Variation in the branching morphologies of clonal plants, fungi, and sessile marine invertebrates is frequently correlated with a suite of life-history traits (e.g. 'phalanx' or 'guerilla'). These correlations have been interpreted to be the causal product of selection. A tacit assumption of selection on a trait is that development is canalized in the manner Waddington originally suggested for aclonal taxa, i.e. small perturbations in development result in a return to an equilibrial morphology. We tested this assumption by manipulating developing colonies of the hydroid *Hydractinia echinata*. The growth trajectory of these colonies follows a clone-specific schedule of production of three structures: feeding polyps, stolonal mat, and peripheral stolons. Isogeneic manipulations of the relative frequency of these structures show that developing colonies can regulate the rate of production of these three structures, but that regulation does not result in rapid convergence on a common growth trajectory.

1. INTRODUCTION

Clonal life cycles are known in plants, animals and fungi and are widely appreciated as displaying patterns in ecology and evolution which differ profoundly from those characterizing aclonal taxa (recent symposium volumes include: Boardman *et al.* 1973; Larwood & Rosen 1979; Jackson *et al.* 1985; Harper 1986; Harper *et al.* 1986; and books include: Harper 1977; Buss 1987; McKinney & Jackson 1989; Caswell 1989). Although differences in demography and life history between clonal and aclonal taxa are pronounced, the substantive intellectual issues with which this symposium is grappling are identical in the two groups; only the life-history traits upon which we focus our attention differ. Yet clonal taxa offer some unique experimental options, precluded in aclonal taxa, which we hope to show are useful in testing tacit assumptions underlying current practice in the field of life-history theory.

Many clonal taxa are substrate-bound, encrusting organisms which advance over a surface by stolons, runners, rhizomes or analogous structures (Harper 1977; Jackson 1979). The vast majority of the fungi are of this habit as are colonial invertebrates in the phyla Porifera, Cnidaria, Bryozoa, as well as certain ascidians, annelids, entoprocts, phoronids, numerous algal groups, virtually all cryptogamic plants, and most herbaceous angiosperms. These groups constitute most of what covers the face of the planet (as opposed to what moves over the surface). In many groups, the morphology can be idealized as being composed of feeding and reproductive entities called ramets (e.g. zooid, polyp, plantlet) which are connected to other ramets, with varying degrees of permanence, by a vascular system (e.g. hyphae, rhizomes, stolons, funiculi). For simplicity, we will refer to all vascular connections as stolons. The spatial arrangement of ramets and stolons, in particular their modes of elongation and branching define a central life-history character of clonal taxa without obvious parallel in aclonal groups. It is on the interpretation of this character that we will focus our attention.

The branching patterns of clonal organisms have been variously interpreted as analogous to foraging behaviour, where the pattern of branching serves to locate patchily distributed resources, or to refuge seeking, where the pattern of branching defines a search strategy for the location of 'safe sites,' or as a competitive mechanism, wherein a pattern of branching reflects differential commitment to defending a particular site (Buss 1979; Jackson 1979; Lovett-Doust 1981; Bell 1984; Harper 1985; Salzmann 1985; Schmid 1985, 1986; Slade & Hutchings 1987; Sutherland 1987; Sutherland & Stillman 1988). Although an exhaustive review of this literature is beyond the scope of this study, an example of this reasoning is perhaps useful. Animal and plant biologists have independently contrasted groups that grow by the production of numerous, closely packed ramets with short vascular connections with groups that produce few, widely spaced ramets on long vascular connections (figure 1). These two ends of a spectrum have been shown to correlate with a variety of other life history characters (table 1).

The reasoning here is that which characterizes most life-history theorizing. One recognizes a life-history trait believed to be shaped by selection and reasons that trade-offs between that trait and other traits may define a suite of characters which can, in principle,

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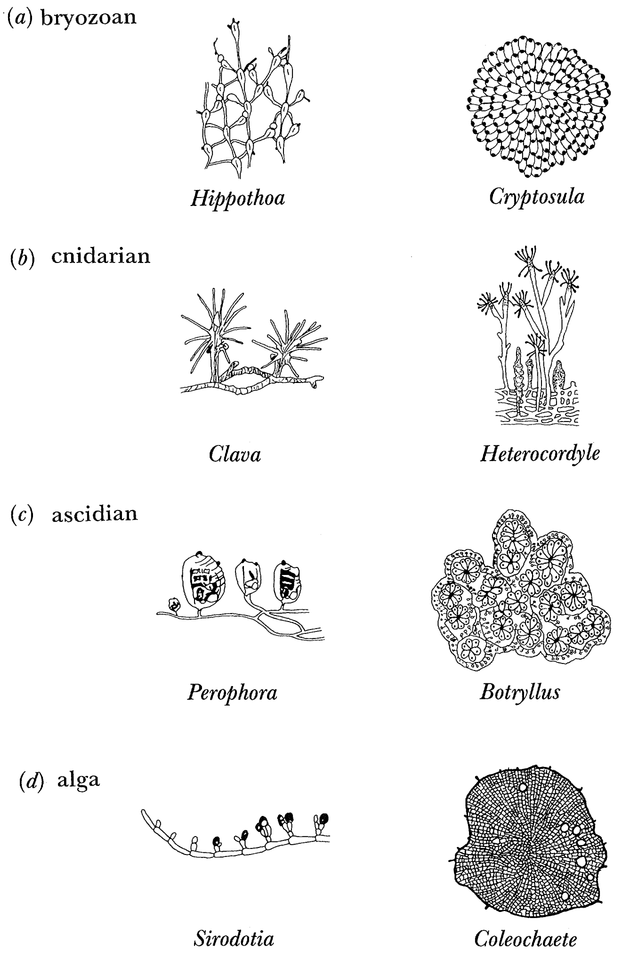

Figure 1. Examples of runner-like and sheet-like forms in (*a*) bryozoans, (*b*) cnidarians, (*c*) ascidians and (*d*) algae. Comparable variation is known in various fungal groups, in vascular plants, in several protist groups, and in sponges.

Table 1. *Comparison of life-history strategies of guerilla and phalanx forms*

life-history trait	phalanx compared with guerilla
size at first reproduction	larger
growth rate	slower
fecundity	lower
capacity for dispersal	lower
commitment to site of recruitment	higher
regenerative potential	higher
intra-colony reallocation of resources	higher
zooid polymorphism	higher
competitive ability	higher
morphological stability	higher

[a] drawn from tables in Buss (1979), Jackson (1979) and Harper (1985).

withstand interactions between it and an environment populated by organisms displaying a different suite of characters (Stearns 1976, 1977). Central to this reasoning is the concept of natural selection shaping an organism's response to trade-offs. While the power of this concept is undoubted, the problems with testing it are manifest: realistic evolutionary experiments can only be done with microbes, quantitative genetic signatures consistent with the operation of natural selection have alternative explanations, and correlations within and between taxa, however repeated, do not imply causality.

We here note that aspects of a selection-based interpretation of clonal organism morphology and life history are based on certain tacit assumptions with regard to the nature of development. Waddington represented development as a problem in dynamic systems theory (i.e. 'the epigenetic landscape'). His 'main thesis is that developmental reactions, *as they occur in organisms submitted to natural selection*, are in general canalized. That is to say, they are adjusted so as to bring about one definite end-result regardless of minor variations in conditions during the course of development' (Waddington 1942, p. 563, emphasis in the original). Thus, small disturbances to a developing organism should result in a return to an equilibrial morphology. To the extent that this conceptualization of the problem is a proper one, one might use adherence to Waddington's predictions as a proxy test for the role of selection in shaping life-history characters.

Let us restate the issue. The growth morphology of a clonal organism is known to be correlated with a suite of other life-history traits (table 1) and it is frequently claimed that this complex of traits is co-adapted. Because perturbations, in the forms of death of ramets, accidental severing of stolons, partial predation, and the like, are inevitable in natural populations, we would expect that a developing clone would respond to perturbations by return to its original morphology. That is to say, if selection is indeed favouring a particular morphology, then selection should act to buffer the development of that organism from those perturbations likely to be encountered (Waddington 1942). If we find that morphology is not canalized, then either our conceptualization regarding the relation between selection and canalization is flawed, or our hypothesis that selection underlies the correlation between life history-traits bears re-examination.

In clonal organisms, where development and growth are inseparable, Waddington's prediction can be directly subjected to an experimental test. We have performed experimental perturbations of developing colonies of the hydroid *Hydractinia echinata*. The growth trajectory of this species follows a clone-specific schedule of production of three structures: feeding polyps (ramets), stolonal mat, and peripheral stolons. Isogeneic manipulations of the relative frequency of these structures show that developing colonies can regulate the rate of production of these three structures, but that regulation does not result in rapid convergence on a common growth trajectory.

2. THE *Hydractinia* SYSTEM

The colonial hydroid *Hydractinia* is a common inhabitant of nearshore waters in temperate and boreal regions worldwide. In the North Atlantic, *Hydractinia echinata* and related species (Buss & Yund 1989) are

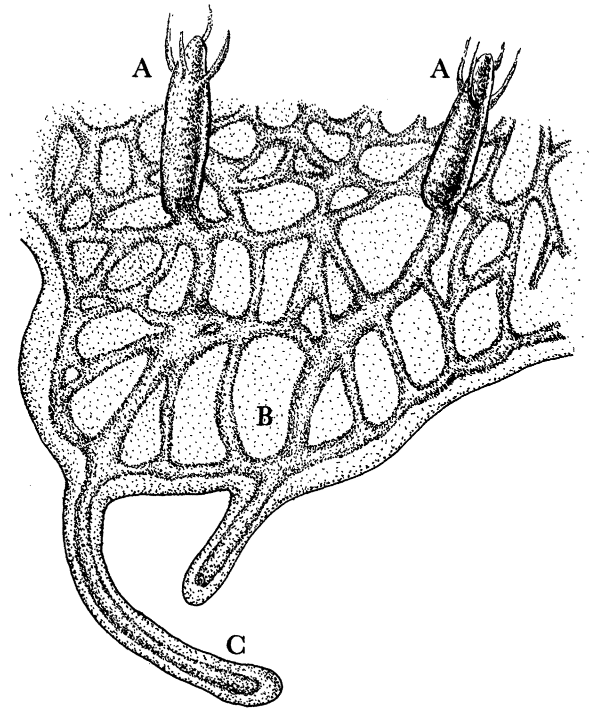

Figure 2. Diagram of a *Hydractinia* colony showing: (*a*) feeding polyps, (*b*) stolonal mat and (*c*) peripheral stolons.

frequently found encrusting gastropod shells inhabited by hermit crabs. The life cycle of *Hydractinia* is simple; dioecious colonies shed gametes, fertilization is external, and the zygote gives rise to a crawling planula larva. The larva settles on a hermit crab shell, metamorphoses into a primary polyp, and grows to give rise to a colony. Asexual propagation from one shell to another is not a common feature of the life cycle.

A *Hydractinia* colony is composed of three distinct structures: polyps, stolonal mat, and peripheral stolons (figure 2). Polyps are feeding organs (gastrozooids), organs of reproduction (gonozooids), and organs of defence (tentacular zooids and dactylozooids). Of these polyp types, only feeding zooids are relevant to the current investigation. Defensive polyps occur only when induced by other organisms, hence do not appear in monospecific laboratory culture. Gonozooids only appear when the limits of a substratum have been reached (or at very large colony size), and these conditions were experimentally precluded. Feeding polyps arise from either stolonal mat or peripheral stolons. Stolonal mat is a closely knit complex of anastomosing endodermal canals capped, except at the mat periphery, with a uniform sheet of ectoderm. Peripheral stolons are continuous with the endodermal tubes of the mat, but extend beyond it.

Growing *Hydractinia* colonies display substantial variation in the relative proportions of these three structures, hence in colony shape (figure 3). Variation ranges from clones that lack peripheral stolons altogether ('mat-type' of Hauenschild 1954) to colonies that bear high proportions of peripheral stolons ('net-type' of Hauenschild 1954). This variation in colony morphology is apparent only in the early stages of ontogeny. As colonies come to cover all available substratum, peripheral stolons develop into stolonal mat. Ontogenetic variation in colony morphology thus largely parallels the between-taxa variation captured by the 'phalanx' versus 'guerilla' distinction (table 1).

Hydractinia is susceptible to the usual range of physical challenges (e.g. desiccation, salinity stress, temperature) and natural enemies (e.g. disease, predators, intra- and interspecific competitors). Of these, only intraspecific competition is known to discriminate between colonies on the basis of colony morphology (Buss *et al.* 1984; Buss & Grosberg 1990). When colonies of *Hydractinia* encounter one another, one of two results may occur; colonies may either fuse or reject. Fusion is limited to close kin and occurs only rarely in natural populations (Hauenschild 1954, 1956; Buss & Shenk 1990). Rejection, too, may take one of two forms – either passive or aggressive (Buss *et al.* 1984; Buss & Grosberg 1990; Buss & Shenk 1990). Passive rejection occurs when two mat-type colonies encounter one another, whereupon both colonies erect a barrier to further cell–cell contact and co-exist. When a net-type colony encounters another colony, an active aggressive response is triggered (Ivker 1972; Buss *et al.* 1984; Lange *et al.* 1989).

The aggressive response has two components: the local proliferation of stolons and the destructive effect of stolons upon one another. Whenever a stolon encounters an incompatible one, a new tip is induced, which may, in turn, induce further tip formation in subsequent encounters (Müller *et al.* 1987). This capacity for local induction of stolonal tips leads to local stolonal proliferation in areas where the stolons of two colonies come into contact. In addition to local proliferation, stolon tips differentiate in the presence of conspecifics. Ultrastructural (Buss *et al.* 1984) and time-lapse video analysis (Lange *et al.* 1989) have documented the migration of nematocytes into stolonal tips, producing swollen (i.e. hyperplastic) stolons. Hyperplastic stolons discharge their nematocysts into neighbouring tissues and effect local destruction (Buss *et al.* 1984; Lange *et al.* 1989). This process continues at points of intraspecific stolonal contact until one colony has annihilated the other, or until all stolons disappear and colonies co-exist.

Since net-type colonies are superior intraspecific competitors, intraspecific variation in *Hydractinia* differs from the usual pattern in this life-history correlate (cf. table 1). Further, since only stolons are capable of giving rise to hyperplastic stolons (Buss *et al.* 1984; Buss & Grosberg 1990), net-type colonies gain this superiority by virtue of their greater amounts of peripheral stolons. Given the multiplicative nature of the tip proliferation, it is not surprising that the absolute amount of peripheral stolon tissue has been found to be an important predictor of competitive ability (Buss & Grosberg 1990).

Several lines of evidence suggest that selection on colony morphology occurs in natural populations:

1. *Variation in morphologically based competitive ability exists and is heritable.* In natural populations, a continuous range of variation, from extreme mat-type to extreme net-type colonies, is found. Analysis of clonal

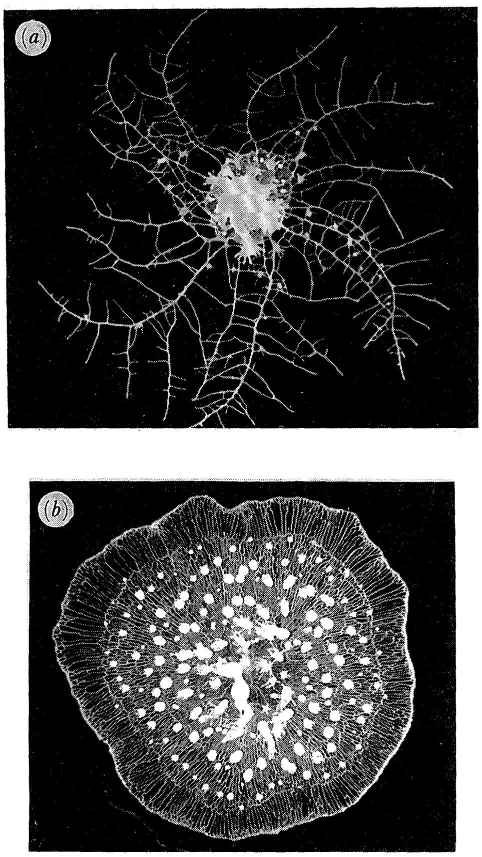

Figure 3. Photographs of a (*a*) 'net-type' and a (*b*) 'mat-type' colony of *Hydractinia*.

repeatibility (i.e. 'common garden experiments') and breeding studies have shown that variation in colony morphology exhibits broad-sense heritability (McFadden *et al.* 1984; Blackstone & Yund 1989; Buss & Grosberg 1990; Blackstone & Buss, 1991; see Falconer, 1981, for general discussion of heritability).

2. *Intraspecific competition occurs chronically in natural populations.* Studies of nine populations in the north-eastern United States have shown that intraspecific competition is a common occurrence in all populations (Yund *et al.* 1987; Buss & Yund 1988; Yund & Parker 1989). In addition, museum specimens spanning the past century display frequencies of intraspecific competition within the range detected in modern populations (Buss & Yund 1988). Finally, limited samples from Miocene populations display signatures of population structure (e.g. size-frequency distributions) known to be driven by intraspecific competition in modern populations (Buss & Yund 1988).

3. *Within and between population variation suggests selection for competitive ability.* The frequencies of colonies of net-type morphology covaries with the frequency of intraspecific competition in between-population comparisons (Yund 1991). Within a given population, the frequency of net-type colonies is enhanced on completely covered shells, which have experienced the selective filter, relative to the frequency of newly recruited colonies (Yund 1991).

4. *Morphological specialization.* The occurrence of a morphology, the hyperplastic stolon, that is specialized for competition is strong *a priori* grounds for suspecting a chronic importance of this process. Notable is the fact that the hyperplastic stolons of *Hydractinia* are known to be induced only upon contact with a competitor and that they are effective only against conspecifics (McFadden 1986).

In summary, *Hydractinia* displays intraspecific variation in colony morphology that mimics the variation displayed between higher taxonomic groups, and this variation is believed to be subject to natural selection in modern and historical populations. Thus, *Hydractinia* should regulate its morphology when perturbed; in particular, the amount of peripheral stolons should be closely regulated.

3. EXPERIMENTAL STUDIES

(*a*) *Methods*

A single clone of *Hydractinia echinata*, derived from a colony collected from Sylt, North Sea, was used in all studies. Clonal explants were made from stock colonies by standard techniques of explanting (McFadden *et al.* 1984). Explants are small regions of stolonal mat (less than 2 mm^2) bearing 3–5 polyps surgically removed from stock colonies. Explants were held affixed to glass microscope slides with a loop of quilting thread. In 2–4 days, explants attached to the slides and the thread was removed. Twenty clonal replicates established in this manner were maintained in 170-gallon (773 l) recirculating aquaria at 15 °C. Colonies were fed to repletion daily with four-day-old *Artemia* nauplii.

All replicates were observed daily with a Wild dissecting scope and the number of polyps counted directly. The outline of the stolonal mat of each colony was traced from a camera lucida projection. In addition, the extent of peripheral stolon development was estimated by determining the area of the complex polygon generated by connecting the endpoints of all free stolon tips from the camera lucida tracing. The area enclosed by stolons and the area of the stolonal mat was calculated by manually digitizing the camera lucida tracing using a Summagraphic bitpad interfaced to an Apple Macintosh microcomputer.

Three replicates were designated as controls and allowed to grow to a size of > 500 polyps without experimental perturbation. The remaining 17 replicates were perturbed from their normal growth trajectory and thereafter left unperturbed until the colony reached a size of > 500 polyps. The 500 polyp threshold was chosen to substantially exceed the size at which colonies experience high frequencies of intraspecific competition in natural populations (Yund *et al.* 1987; Buss & Yund 1988; Yund & Parker 1989). Perturbations were effected by surgically removing polyps, peripheral stolons or stolonal mat with a microscalpel. Perturbations were chosen in attempt to sample as fully as practical the entire range of mat–polyp–stolon 3-space.

Assessment of the effects of surgery per se is critical to the interpretation of these experiments. *Hydractinia* repairs surgical injury within seconds. The endoderm of the stolonal vasculature is populated by contractile vacuoles (Schierwater *et al.* 1991), which permit rapid contraction of stolons at the site of injury. To insure, however, that the subsequent growth trajectory of a manipulated colony was not governed by the manipulation itself, we perturbed two replicates to point in the mat–polyp–stolon 3-space within the range of variation displayed by unmanipulated control colonies. If surgical effects governed subsequent growth, then these colonies may be expected to deviate from that observed for control colonies.

(*b*) *Analysis*

Operationally, we defined regulation as the response of a manipulated colony which differs from that of a control colony and serves to return the manipulated colony to within the range of the control growth trajectory. The experimental protocol perturbed the growth of the manipulated colonies in state space. In response, the manipulated colony could (i) grow all structures, manipulated and unmanipulated, at the same rates as the unperturbed, control colonies; (ii) grow the manipulated (reduced) structure(s) at an accelerated rate compared to the control colonies, or (iii) grow the reduced structure(s) at an accelerated rate and the unmanipulated structure(s) at a reduced rate compared to the control colonies. Using our operational definition, (ii) or (iii) would constitute regulation.

If regulation occurs, it can produce convergence of control and manipulated growth trajectories in rate or in state space. The latter can be assessed by comparing growth trajectories for structures of control and manipulated colonies on arithmetic axes. Similarly, regulation of rates can be assessed by comparing growth trajectories for control and manipulated colonies on log-transformed axes, in which slopes represent the relation between the specific growth rates of the structures in question (this follows from the derivative form of the allometry equation; for examples, see Blackstone (1987), Blackstone & Yund (1989)).

Specific growth rates were used as measures of growth rate. Each rate was calculated as increment in area or number (area for stolonal mat and peripheral stolon, number for polyp) per time increment per initial area or number. Although 'specific' technically refers to 'divided by mass', any measure of size can be used, as a specific growth rate has units of 1/time. This rate approximates the derivative with respect to time of the logarithm of the size measure and is a physiologically meaningful measure of rate (see, for example, Stebbing 1981). For each of the ten days following the surgical manipulation, specific growth rates for polyps, stolonal mat, and peripheral stolons were calculated (in some cases data were available for 11 daily intervals). Thus, the growth of a single colony was assessed by ten (or in some cases 11) specific growth rates. Using analysis of variance, the rates of stolon, mat, and polyp production were compared between control and manipulated colonies; using multivariate analysis of variance (MANOVA), the relations among these rates were compared between control and manipulated colonies.

Growth trajectories were compared visually and with analysis of covariance; because competition focuses on the area of peripheral stolons, this area was treated as the outcome variable. The correlations of polyp, mat, and stolon generally exceed 0.95; this colinearity rendered multiple regression of little use.

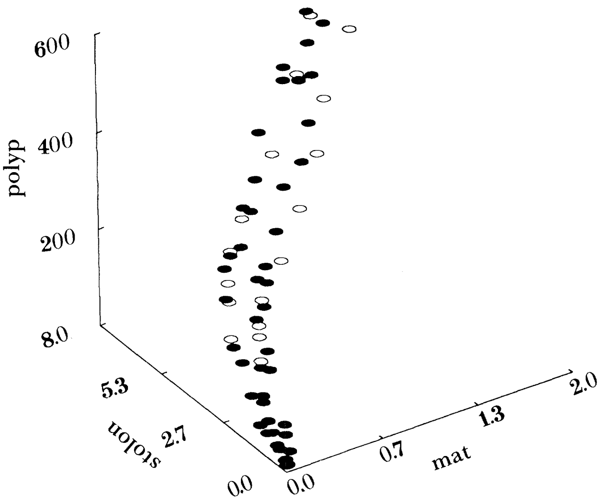

Figure 4. Three-dimensional scatterplot of polyp number, stolonal mat and peripheral stolon area (cm^2) for unmanipulated colonies (closed circles) and colonies manipulated to approximate points in the control growth trajectory (open circles).

(*c*) *Results*

We do not attempt here to utilize the growth trajectories of perturbed colonies to construct equations of state for developing *Hydractinia* colonies. Rather we will seek only to provide a qualitative description of the behaviour of perturbed colonies. We will ask only whether there is evidence for regulation and convergence of rates, states, or both, between control and manipulated colonies.

Controls. For two control manipulations (i.e. colonies that were surgically manipulated, but not displaced from the control trajectory in state space) and two unperturbed colonies over the ten-day period following the manipulations, there was no significant difference in the rate of production of polyps ($F = 2.38$, d.f. $= 1{,}38$, $p > 0.10$), stolonal mat ($F = 0.98$, d.f. $= 1{,}38$, $p > 0.30$), or peripheral stolons ($F = 0.03$, d.f. $= 1{,}38$, $p > 0.80$), and the relation among these rates does not differ between control and manipulated colonies (MANOVA, approximate $F = 1.23$, d.f. $= 3{,}36$, $p > 0.30$). The growth trajectories of unmanipulated and control colonies are shown in figure 4.

Regulation. Perturbed *Hydractinia* colonies exhibit regulation of specific growth rates. Examples of this regulation will be provided for three representative classes of manipulations performed after two weeks of growth.

1. Peripheral stolon area reduced (50%), mat and polyps undisturbed. Polyp growth was unchanged ($F = 0.05$, d.f. $= 1{,}19$, $p > 0.80$), stolonal mat growth was unchanged ($F = 0.79$, d.f. $= 1{,}19$, $p > 0.35$), while peripheral stolon growth was accelerated ($F = 6.94$, d.f. $= 1{,}19$, $p < 0.05$) in one manipulated colony

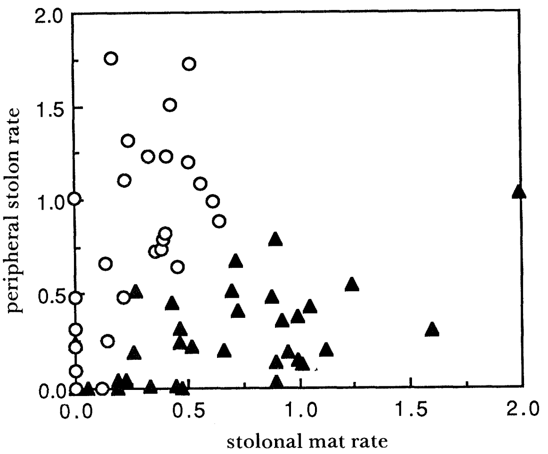

Figure 5. Bivariate scatterplot of specific growth rates ($1/10^2$/h) for peripheral stolon and stolonal mat areas from three control colonies (triangles) and three colonies in which polyp number and peripheral stolon area were reduced by 50 and 75% respectively (circles). Specific growth rates are for the ten days following the manipulations; while polyp growth rate was unchanged, note that the manipulated colonies exhibit not only an acceleration of peripheral stolon growth, but a slowing of stolonal mat growth.

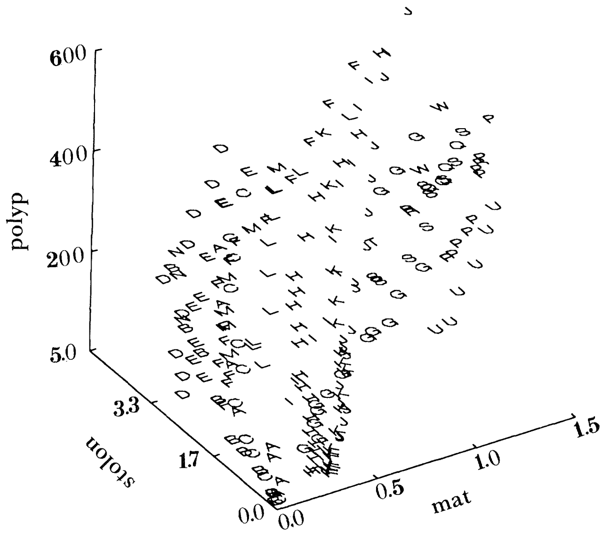

Figure 6. Three-dimensional scatterplot of polyp number, stolonal mat area (in square centimetres) and peripheral stolon area (in square centimetres) for control and all manipulated colonies. Each colony is represented by a different letter and each sequence of the same letter represents the growth trajectory of that colony at daily intervals.

compared to one unmanipulated control over the same ten-day time period. The relation among rates was altered as well (MANOVA, approximate $F = 6.41$, d.f. = 3,17, $p < 0.01$), although this is likely to reflect the acceleration of the peripheral stolon growth in the manipulated colony rather than more complex regulation.

2. Peripheral stolon area reduced (75%), polyp number reduced (50%) and mat left undisturbed. For three manipulated colonies compared to three unmanipulated controls over the same time period, polyp growth was unchanged ($F = 0.33$, d.f. = 1,60, $p >$ 0.55), stolonal mat growth was slowed ($F = 21.77$, d.f. = 1,60, $p \ll 0.001$), and peripheral stolon growth was accelerated ($F = 19.03$, d.f. = 1,60, $p \ll 0.001$) over the same ten-day time period. A highly significant MANOVA (approximate $F = 25.4$, d.f. = 3,58, $p \ll$ 0.001) reflects the dramatic changes in the relation of stolonal mat and peripheral stolon growth rates in these manipulations (figure 5).

Although polyp growth rate was unchanged over the ten-day period, it should be noted that polyp growth rate was extremely rapid for the 24-hour period immediately following the manipulation (0.027 polyps polyp^{-1} h^{-1} versus 0.011 for control colonies, $F = 7.74$, d.f. = 1,4, $p < 0.05$). After perturbation, the colony produces new polyp buds, generally in the same location of those removed, and after this period of formation of polyp buds, polyp growth returned to the control value ($F = 2.00$, d.f. = 1,4, $p > 0.20$).

3. Polyp number reduced (> 50%), mat area reduced (> 50%), and peripheral stolons left undisturbed. For two manipulated colonies compared to two unmanipulated controls over the same time period, polyp growth accelerated slightly ($F = 4.21$, d.f. = 1,40, $p < 0.05$), stolonal mat growth accelerated slightly ($F = 5.10$, d.f. = 1,40, $p < 0.05$), whereas peripheral stolon growth was unchanged ($F = 0.08$, d.f. = 1,40, $p > 0.75$). The relation among rates was altered as well (approximate $F = 2.98$, d.f. = 3,38, $p <$ 0.05).

The patterns displayed by these three examples appear to be generally true; all three structures tend to exhibit a regulatory response in growth rate when perturbed. Further, both the reduction of growth of unmanipulated structures as well as the acceleration of growth of manipulated structures can occur (figure 5), suggesting a somewhat complex colony-wide regulation of growth.

Convergence onto control trajectories. Perturbed *Hydractinia* colonies exhibit regulation of rates, but regulation is not sufficiently rapid to return colonies to the control growth trajectory in mat–polyp–stolon space. Figure 6 shows the trajectories of all disturbed colonies. It is apparent that regulation does not result in a rapid attainment of control values. Using the representative manipulations introduced above, we examine the relation between control and perturbed colonies in more detail. For the ranges relevant to this analysis, the relations between both arithmetic and log-transformed values of stolonal mat and peripheral stolon areas are linear, at least to a first approximation. Thus, for both rates and states, differences between control and manipulated trajectories can be examined using analysis of covariance. In particular, convergence of trajectories is apparent from heterogeneity of slopes, i.e. the presence of a significant interaction in the analysis.

1. Peripheral stolon area reduced (50%), mat and polyps undisturbed. In this case, the arithmetic values (peripheral stolon area as the outcome, stolonal mat area as the covariate) show a non-significant interaction between control and manipulated colonies ($F = 0.43$, d.f. = 1,22, $p > 0.50$), but nevertheless the control and manipulated trajectories differ strongly in

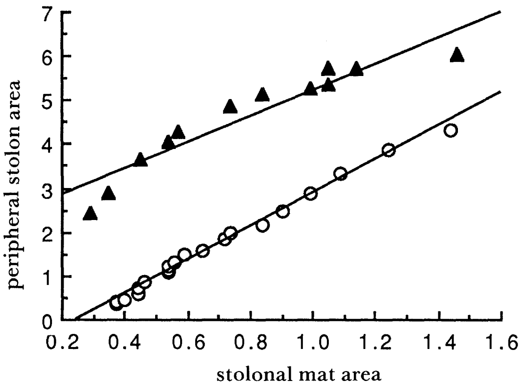

Figure 7. Bivariate scatterplot of stolonal mat and peripheral stolon area (in square centimetres) for a control colony (triangles) and a colony in which stolonal area was reduced (circles). Lines represent least-squared regressions.

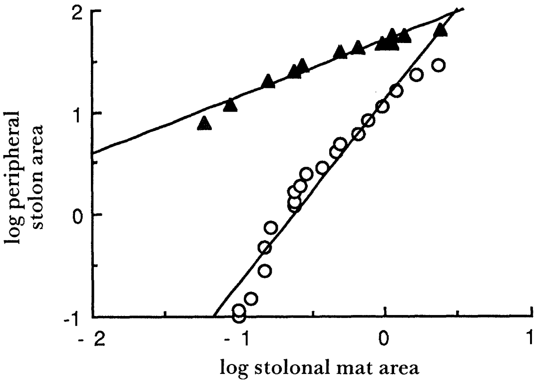

Figure 8. Bivariate scatterplot of log-transformed values of stolonal mat and peripheral stolon area for the control colony (triangles) and the manipulated colony (circles) from figure 7. Lines represent least-squared regressions.

elevation ($F = 286$, d.f. $= 1{,}23$, $p \ll 0.001$). Thus, these trajectories are distinct and not converging. On the other hand, analysing the log-transformed values shows a highly significant interaction ($F = 57.25$, d.f. $= 1{,}22$, $p \ll 0.001$), and the control and manipulated trajectories converge by virtue of this difference in slope. Note that this heterogeneity of slopes is analogous to the difference in specific growth rates discussed above (see Blackstone 1987; Blackstone & Yund 1989).

2. Peripheral stolon area reduced (75%), polyp number reduced (50%) and mat left undisturbed. Each of these manipulated colonies are similar to the colony discussed above, e.g. for one colony and one control, figures 7 and 8 show the arithmetic and log-transformed trajectories. For arithmetic values, there is a significant interaction term ($F = 6.24$, d.f. $= 1{,}29$, $p < 0.05$); for log-transformed values the interaction is highly significant ($F = 101$, d.f. $= 1{,}29$, $p \ll 0.001$). Although trajectories converge in both rate and state space, convergence of rates is much stronger. Further, this convergence is due to both an increase in the rate of peripheral stolon growth and to a decrease in stolonal mat growth.

3. Polyp number reduced (> 50%), mat area reduced (> 50%) and peripheral stolons left undisturbed. Both of these manipulated colonies exhibit the same patterns as those seen in the above examples; weak regulation of states and strong regulation of rates. For instance, for one of the manipulated colonies and one control, the arithmetic values for peripheral stolon and stolonal mat area show a significant interaction ($F = 5.90$, d.f. $= 1{,}14$, $p < 0.05$), whereas the log-transformed values show a much stronger interaction ($F = 98$, d.f. $= 1{,}14$, $p \ll 0.001$).

In summary, the experimental manipulations of *Hydractinia* colonies provide some interesting results; although growth rates of manipulated (reduced) structures are often accelerated (and growth rates of unmanipulated structures are sometimes slowed, figure 5), and although the relations of growth rates rapidly converge on control trajectories (figure 8), this regulation does not produce convergence on control growth trajectories in state space (figures 6 and 7).

4. DISCUSSION

(*a*) *Is the* Hydractinia *result generalizable?*

Our results clearly show that *Hydractinia* exhibits regulation of growth rates, but that regulation is not sufficiently rapid for colonies to re-establish control growth trajectories following disturbance. One might reasonably ask whether this result is likely to be generally true for clonal organisms or whether it is peculiar to this hydroid. Among benthic hydroids, *Hydractinia* has long been recognized as the pinnacle of colony integration (Mackie 1973, 1986). The anatomy of its vascular system is far more sophisticated than that of most other hydroids, in terms of the number and types of stolonal anastomosis that occur and, hence, *Hydractinia* would be among the hydroids most likely to display regulation. Comparisons between taxa are more difficult. Some clonal taxa may well display more effective regulation. Bryozoan zooids communicate through funiculi that are clearly less efficient, in terms of transport rates, than is the colonial vasculature of hydroids (Best & Thorpe 1985). Nevertheless, the rigid zooidal architecture of these forms and the need of some colonies to generate communal feeding currents may limit the range of permissible deviations from a given colony form. In most other clonal groups, however, ramets are only loosely connected by stolons and display far more restricted sharing of resources through ramets than is the case in *Hydractinia* (Pitelka & Ashmun 1985). Control of the distribution of resources is often localized to individual ramets or small numbers of nearby ramets. In any such clone, one may suspect that perturbations of these systems will generate results similar to those we report here.

(*b*) *The application of Waddington's concept to clonal taxa*

Waddington was clearly thinking of aclonal organisms in formulating his conclusions with respect to canalization. This is particularly evident in his concentration on the end-product of a developmental sequence. Unlike aclonal organisms, development in clonal organisms is continuous and few clonal organisms come to adopt a fixed end-product. Perhaps it is a disservice to Waddington's memory to apply his notions to clonal organisms. We think not. The development of any organism can be approached as a dynamical systems problem. More importantly, Waddington's central point is that canalization should occur in any trait subject to selection and this argument is not sensitive to the choice of organism.

(*c*) *Interpretation of experimental findings*

Given that Waddington's argument is applicable to this case, there are several possible interpretations of our findings.

The adaptation interpretation. In both the modern and historical populations we have studied (Yund *et al.* 1987; Buss & Yund 1988; Yund & Parker 1989; Yund 1991), the vast majority of competitive interactions occur as pairwise encounters. Most encounters occur at small colony size ($\ll 50$ polyps) by virtue of the fact that colonies preferentially recruit to sites on the inner lip of the aperture of a shell. Such encounters are rapidly resolved in favour of one colony or another while colonies are still of small size (Yund *et al.* 1987). Given the dependence of competitive ability on the quantity of stolonal tissue and the multiplicative relation between tip proliferation and initial tip number in aggressive interactions, one would expect that rapid regulation of state would be strongly favoured by selection. Yet, regulation of state was not observed; the observed regulation of rates is insufficient to return a colony to pre-disturbance morphology (figure 6).

Nevertheless, selection may have produced canalized rates of increase of stolons, i.e. hyperplastic stolon production depends on both the initial number and on the rate of tip production. Given the slow convergence of manipulated colonies onto control growth trajectories, a high rate of stolon production will likely be favoured only in the small percentage of competitive interactions that occur at large colony size. Such interactions are found when one colony recruits to the site near the aperture of the shell and another recruits to as site distant from the lip of the aperture, most commonly in the siphonal notch (Yund *et al.* 1987; Buss & Yund 1988; Yund & Parker 1989).

The non-adaptation interpretation. The observed regulation of rates is not sufficiently rapid to support an adaptationist interpretation for the most common selective environment, that of pairwise encounters at small colony size. In such contests, initial stolon number is crucial, since the encounter is too brief for rate of production to compensate for an initial disadvantage. Perhaps *Hydractinia* colonies do not rapidly regulate mat–polyp–stolon state because they cannot; they lack appropriate genetic variation for selection to work upon. In this view, selection is potentially efficacious in all matters except rapid canalization, and whereas the most common selective environment favors rapid regulation of state, this is impossible and the organism persists despite this defect.

The structuralist interpretation. A developing colony may, in principle, be characterized by a series of equations defining state and transitions in state. At present, such descriptions are lacking for any organism. It is reasonable to ask, however, what would be the range of dynamical behaviour displayed by such a description? Goodwin and others (Goodwin 1982; Goodwin & Saunders 1989; Goodwin *et al.* 1989) have noted that it is conceivable that the underlying dynamics of a system may be competent to produce observed patterns without appeal to the action of natural selection. Perhaps it is the case that correlations, say between life-history traits, are *necessarily* produced by features of the underlying dynamics. If this is the case, then the fact that natural selection favors these correlations does not necessarily imply that these correlations have been shaped by natural selection.

Consider the effect of a disturbance on a *Hydractinia* colony. Stolons are essentially tubes for the transport of the hydroplasm. This fluid is propelled by contraction of polyps and stolons elongate most rapidly when flow is directed into their tips (Wyttenbach 1973). Polyps are connected at their bases to several stolons. A particular polyp may be connected to both narrow stolons of the stolonal mat and wide stolons leading through the stolonal mat to peripheral stolons. In one of our representative examples, disturbances were made to both peripheral stolons and polyps. The removal of polyps resulted in a replacement by new, smaller polyps. Thus, the capacity of polyps to propel a given volume of fluid is reduced after a disturbance. Because peripheral stolons are wider than stolons of the mat, these stolons will receive a greater volumetric rate of flow than stolons in the mat, because flow rate is proportional to the radius. Thus, after the disturbance the peripheral stolons will elongate at faster absolute rates than do stolons in the mat. As a purely physical consequence, then, one would expect that the relative rate of stolon growth will be accelerated and rate of mat growth be depressed in a disturbance of this sort. This pattern of 'regulation' is just what is observed in such manipulations (figure 5).

Although we are far from a dynamical system portrait of the hydrodynamics of colony form in *Hydractinia*, such a description may generate the pattern of regulation of rates that we have observed. Should this be the case, one interpretation of our experimental findings is that these hydrodynamical considerations are but the proximate basis for the ultimate pattern produced by selection. The structuralist interpretation is not, however, a claim about proximate explanations; it is a claim that ultimate explanations are unnecessary for certain classes of proximate claims. The general distinction may be stated in terms of the *Hydractinia* problem. Imagine that the equations defining hydroid growth in terms of the underlying hydrodynamics provide multiple solutions; say, colonies may display either regulation of rates or no regulation of rates. Then the claim that selection has chosen among these solutions to favour regulation of rates is necessary. If, however, one finds that the *only* solution to equations defining these hydrodynamics is that of regulation of rates, then a claim for the efficacy of selection is neither necessary nor sufficient.

The problem here is one of null models. Selection is a process that acts upon existing variation. We do not know the generating functions that produce that variation and can only gain a highly restricted glimpse of the dynamical potential by assessment of existing variation. Hence to rigorously assess whether selection is efficacious, or even necessary, we must first know the range of permissible dynamics. These must constitute the null model against which we assess the role of selection. Note that this structuralist position is not at variance with the non-adaptation interpretation, but that its claims are more pervasive. The non-adaptation interpretation permits selection to be efficacious in all cases in which appropriate genetic variation occurs. The structuralist perspective is more demanding in that it requires first a complete understanding of the range of possible dynamical behaviours before assessment can be made of whether selection is necessary to account for patterns we observe.

With our present understanding of this system, we cannot say which of these three interpretations is correct, or even most likely to be correct. We do, however, strongly advocate the view that the latter two interpretations are as worthy of attention as the former.

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REFERENCES

Bell, A. D. 1984 Dynamic morphology: a contribution to plant population ecology. In *Perspectives on plant population ecology* (ed. R. Ditzo & J. Sarukán), pp. 48–65. Sunderland, Massachusetts: Sinauer Associates.

Best, M. A. & Thorpe, J. P. 1985 Autoradiographic study of feeding and the colonial transport of metabolites in the marine bryozoan *Membranipora membranacea*. *Mar. Biol.* **84**, 295–300.

Blackstone, N. W. 1987 Specific growth rates of parts in a hermit crab: a reductionist approach to the study of allometry. *J. Zool., Lond. A* **211**, 531–545.

Blackstone, N. W. & Yund, P. O. 1989 Morphological variation in a colonial marine hydroid: a comparison of size-based and age-based heterochrony. *Paleobiology* **15**, 1–10.

Blackstone, N. W. & Buss, L. W. 1991 Shape variation in hydractiniid hydroids. *Biol. Bull.* **180**. (In the press.)

Boardman, R. S., Cheetam, A. H. & Oliver, W. A. 1973 *Animal colonies*. Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross.

Buss, L. W. 1979 Habitat selection, directional growth, and spatial refuges: why colonial animals have more hiding places. In *Biology and systematics of colonial organisms* (ed. G. Larwood & B. Rosen), pp. 459–477. London: Academic Press.

Buss, L. W. 1987 *The evolution of individuality*. Princeton University Press.

Buss, L. W. & Grosberg, R. K. 1990 Morphogenetic basis for phenotypic differences in hydroid competitive behaviour. *Nature, Lond.* **343**, 63–66.

Buss, L. W., McFadden, C. S. & Keene, D. R. 1984 Biology of hydractiniid hydroids. 2. Histocompatibility effector system/competitive mechanism mediated by nematocyst discharge. *Biol. Bull.* **167**, 131–158.

Buss, L. W. & Shenk, M. A. 1990 Hydroid allorecognition regulates competition at both the level of the colony and the level of the cell lineage. In *Defense molecules* (ed. J. J. Marchalonis & C. Reinisch), pp. 85–106. New York: Wiley–Liss.

Buss, L. W. & Yund, P. O. 1988 A comparison of modern and historical populations of the colonial hydroid *Hydractinia*. *Ecology* **69**, 646–654.

Buss, L. W. & Yund, P. O. 1989 A sibling species group of *Hydractinia* in the northeastern United States. *J. mar. biol. ass. U.K.* **69**, 857–875.

Caswell, H. 1989 *Matrix population models*. Sunderland, Massachusetts: Sinauer Associates.

Falconer, D. S. 1981 *Introduction to quantitative genetics*. London: Longman.

Goodwin, B. C. 1982 Development and evolution. *J. theor. Biol.* **97**, 43–55.

Goodwin, B. C. & Saunders, P. 1989 *Theoretical Biology: epigenetic and evolutionary order from complex systems*. Edinburgh University Press.

Goodwin, B. C., Sibatani, A. & Saunders, P. T. 1989 *Dynamic structures in biology*. Edinburgh University Press.

Harper, J. L. 1977 *The population biology of plants*. New York: Academic Press.

Harper, J. L. 1985 Modules, branches, and the capture of resources. In *Population biology and evolution of clonal organisms* (ed. J. B. C. Jackson, L. W. Buss & R. E. Cook), pp. 1–33. New Haven, Connecticut: Yale University Press.

Harper, J. L. 1986 *Modular organisms: case studies of growth and form*. London: Royal Society.

Harper, J. L., Rosen, B. R. & White, J. 1986 *The growth and form of modular organisms*. London: Royal Society.

Hauenschild, V. C. 1954 Genetische and entwicklungphysiologische Untersuchungen uber Intersexualitat und bewebevertraeglichkeit bei *Hydractinea echinata* Flem. *Arch. Entwicklungsmech. Org.* **147**, 1–41.

Hauenschild, V. C. 1956 Uber die Vererbung einer Gewebevertraglichkeitseigenschaft bei dem Hydroidpolypen *Hydractinea echinata*. *Z. Naturforsch.* **11b**, 132–138.

Ivker, F. B. 1972 A hierarchy of histoincompatibility in *Hydractinia echinata*. *Biol. Bull.* **143**, 162–174.

Jackson, J. B. C. 1979 Morphological strategies of sessile animals. In *Biology and systematics of colonial organisms* (ed. G. Larwood and B. Rosen), pp. 499–556. London: Academic Press.

Jackson, J. B. C., Buss, L. W. & Cook, R. E. 1985 *Population biology and evolution of clonal organisms*. New Haven, Connecticut: Yale University Press.

Lange, R., Plickert, G. & Müller, W. A. 1989 Histocompatibility in a low invertebrate. *Hydractinia echinata*: analysis of the mechanism of rejection. *J. exp. Zool.* **249**, 284–292.

Larwood, G. & Rosen, B. R. 1979 *Biology and systematics of colonial organisms*. London: Academic Press.

Lovett-Doust, L. 1981 Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. *J. Ecol.* **69**, 743–755.

Mackie, G. O. 1973 Coordinated behavior in hydrozoan colonies. In *Animal colonies* (ed. R. S. Boardman, A. H. Cheetam & W. A. Oliver, Jr), pp. 95–106. Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross.

Mackie, G. O. 1986 From aggregates to integrates: physiological aspects of modularity in colonial animals. *Phil. Trans. R. Soc. Lond.* B **313**, 175–196.

McFadden, C. S. 1986 Laboratory evidence for a size-refuge in competitive interactions between the hydroids

Hydractinia echinata (Fleming) and *Podocoryne carnea* (Sars). *Biol. Bull.* **171**, 161–174.

McFadden, C. S., McFarland, M. & Buss, L. W. 1984 Biology of hydractiniid hydroids. 1. Colony ontogeny in *Hydractinia echinata*. *Biol. Bull.* **166**, 54–67.

McKinney, F. & Jackson, J. B. C. 1989 *Bryozoan evolution.* Boston: Unwin Hyman.

Müller, W. A., Hauch, A. & Plickert, G. 1987 Morphogenetic factors in hydroids: 1. Stolon tip activation and inhibition. *J. exp. Zool.* **243**, 111–124.

Pitelka, L. F. & Ashmun, J. W. 1985 Physiology and integration of ramets in clonal plants. In *Population biology and evolution of clonal organisms* (ed. J. B. C. Jackson, L. W. Buss & R. E. Cook), pp. 399–436. New Haven, Connecticut: Yale University Press.

Salzmann, A. G. 1985 Habitat selection in a clonal plant. *Science, Wash.* **228**, 603–604.

Schierwater, B., Piekos, B. & Buss, L. W. 1991 Hydroid stolonal contractions mediated by contractile vacuoles. *J. exp. Biol.* (In the press.)

Schmid, B. 1985 Clonal growth in grassland perennials. III. Genetic variation and plasticity within populations of *Bellis perennis* and *Prunella vulgaris*. *J. Ecol.* **73**, 819–830.

Schmid, B. 1986 Spatial dynamics and integration within clones of grassland perennials with different growth form. *Proc. R. Soc. Lond.* B **228**, 173–186.

Slade, A. J. & Hutchings, M. J. 1987 Clonal growth and plasticity in foraging behavior in *Glechoma hederacea*. *J. Ecol.* **75**, 1023–1036.

Stearns, S. C. 1976 Life-history tactics: a review of the ideas. *Q. Rev. Biol.* **51**, 3–47.

Stearns, S. C. 1977 The evolution of life-history traits: a critique of the theory and a review of the data. *A. Rev. Ecol. Syst.* **8**, 145–171.

Stebbing, A. R. D. 1981 The kinetics of growth control in a colonial hydroid. *J. mar. biol. Ass. U.K.* **61**, 35–63.

Sutherland, W. J. 1987 Growth and foraging behavior. *Nature, Lond.* **330**, 18–19.

Sutherland, W. J. & Stillman, R. A. 1988 The foraging tactics of plants. *Oikos* **52**, 239–244.

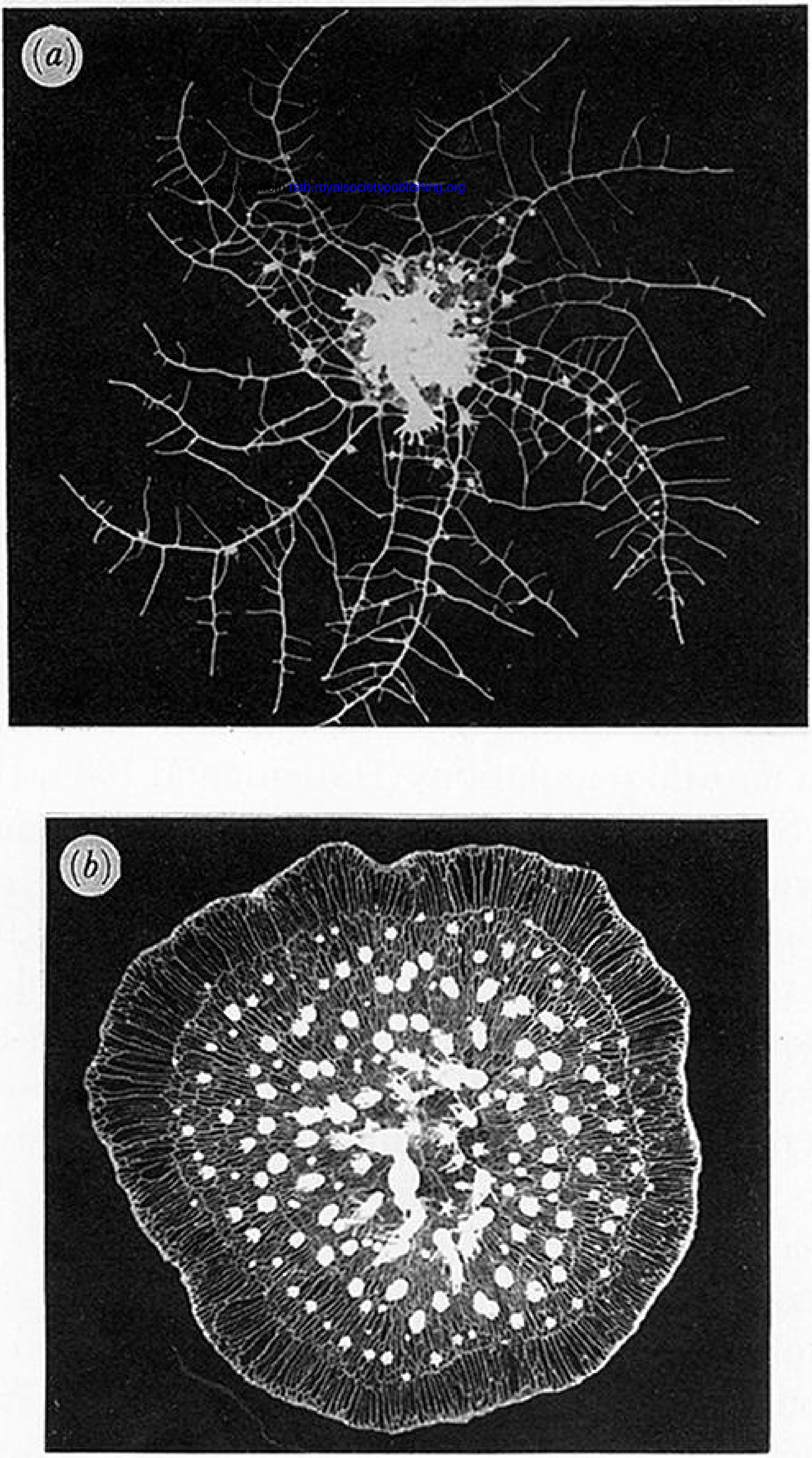
Waddington, C. H. 1942 Canalization of development and the inheritance of acquired characteristics. *Nature, Lond.* **150**, 563–565.

Wyttenbach, C. R. 1973 The role of hydroplasmic pressure in stolonic growth movements in the hydroid, *Bougainvilla*. *J. exp. Zool.* **186**, 79–90.

Yund, P. O. 1991 Natural selection on colony morphology by intraspecific competition. *Evolution.* (In the press.)

Yund, P. O., Cunningham, C. W. & Buss, L. W. 1987 Recruitment and post-recruitment interactions in a colonial hydroid. *Ecology* **68**, 971–982.

Yund, P. O. & Parker, H. 1989 Population structure of the colonial hydroid *Hydractinia* sp. nov. C in the Gulf of Maine. *J exp. mar. Biol. Ecol.* **125**, 63–82.



gure 3. Photographs of a (*a*) ‘net-type’ and a (*b*) ‘mat-type’ colony of *Hydractinia*.