

Evolution of mitotic cell-lineages in multicellular organisms

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Adaptive evolution in multicellular organisms is generally assumed to occur through natural selection acting differentially among the phenotypes programmed by sexually-generated zygotic genotypes. Under this view, only genetic changes in the gamete-zygote-germline-gamete cycle are considered relevant to the evolutionary process. Yet asexuality – production of progeny through proliferation of mitotic cell-lineages – is found in over one half of all eukaryotic phyla, and is likely to contribute to adaptive changes, as suggested by recent evidence from both animals and plants. Adaptive changes in mitotic lineages can be reconciled with contemporary evolutionary thought by fully abandoning the weismannian concept of individuality.

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Mitotic cell-lineages in multicellular organisms can evolve through selection among cells with different genotypes. The possibility that this process leads to adaptive evolution at the level of the multicellular organism has frequently been dismissed on the theoretical basis that evolution (gene frequency changes between ancestors and descendants) requires a lifecycle which either (1) encompasses meiosis and zygote formation (the genet-centered view), or (2) at least starts from a single-celled stage (the ontogenetic view). One or both of these prerequisites is violated in progeny formed from meristematically-derived cell-lineages in plants, in mitotically-derived progeny of many insects and crustaceans, and in many other kinds of vegetative propagules.

The genet-centered view

The genet-centered view holds that an individual (a genet) is the ontogenetic product of a zygote. While it is recognized that mosaicism (the presence of mutational variants among the mitotic cell lineages derived from the same zygote) may have evolutionary consequences, this is often accepted only if somatic mutations are transferred to new zygotes: 'If somatic mutations can be acquired by the gametophytes, they can be passed to future generations, and they have the potential to influence the rates of evolution of plants'¹. Emphasis on sexual reproduction is also seen in the literature on clonal animals, for example the cladoceran *Daphnia magna* 'were produced sexually thereby providing the genetic variants needed for natural selection'².

In short, under this view, meiosis is a

tacit prerequisite for evolutionary change. Incorporation of somatic mutants into gametes is seen as the way in which these mutations can produce new entities capable of evolution by natural selection.

The ontogenetic view

The ontogenetic view holds that, for complex adaptations to evolve, the life cycle of a multi-cellular organism has to be recurrently contracted to a one-celled stage (sometimes this is qualified to allow oligocellular stages). Only then can an ontogenetic programme be gradually changed by selection such that complexity evolves from simplicity, refined adaptation from less refined, elaborate life-cycles from less elaborate.

This view is clearly spelled out by Bonner³ and Dawkins⁴. Significantly, it is also implicit in much of the literature on clonal plants⁵⁻⁷. Thus it defines an individual as the ontogenetic product of a single cell. Therefore, budding-off of medusae in cnidarians, runners in *Fragaria* (strawberry) or fission in *Lemna* (duckweed) do not produce new individuals in this scheme, because the origins of the new entities are multicellular. Under the ontogenetic view, such new entities are no more than growth of the parent, despite their being functionally and physically distinct. Indeed, by this argument, all 'Cox's orange pippin' apple trees in the world represent nothing but continuing growth since 1825 of just one individual on several different continents!

Combining the ontogenetic and genet-centered views

By positioning these two definitions of an organism into a 2 × 2 matrix, we can

illustrate critical consequences of applying the views above (Fig. 1). Three important aspects of evolutionary thought coincide in the upper left element of the matrix. First, the ontogenetic and genetic definitions of individuality overlap in this element, since a zygote is always one-celled. Second, prominent model organisms for geneticists and developmental biologists (such as *Drosophila*, *Xenopus*, *Mus* and *Gallus*) fall in that element, as do the bird and mammal species so favoured for study by evolutionary ecologists. Third, the weismannian doctrine (Box 1) holds true only in this element. This congruence between dominant lines of thought and dominant experimental programmes has directed much of contemporary evolutionary thinking.

Importantly, a substantial proportion of life falls outside the top left element of the matrix. In the lower-left element we find life-histories that conform to the ontogenetic but not the genet-centered definition of individuality: aphids and all other animals in which mitotically-derived progeny are regularly produced from just one cell.

Meristematically-derived ferns also fall in this element, since fern apical meristems consist of just one cell⁸. In the lower-right element, on the other hand, we find life-histories encompassing buds, sports, runners and all other forms of vegetative reproduction which are prevalent among seed plants; analogous modes also occur in many animals¹¹. These processes form physically and physiologically independent progeny, by budding-off of multicellular meristematic cell colonies (or equivalent structures in animals). Indeed, it can be argued that all seed plants qualify for inclusion in this element, since all such plants consist of modules that are to varying extents physiologically independent of each other. For the present purposes, we consider only modules that are physically disconnected from the parent plant; the different branches and twigs on a tree do, after all, share a common stem and root system.

Individuality and evolution by natural selection

The definitions of individuality inherent in the ontogenetic and genet-centered views above are only two of a set of conceivable definitions. Dismissal of other definitions^{5,7} may frustrate our appreciation of valid evolutionary processes. As suggested by Hull¹², all that is needed for evolution by natural selection is that there be descendent entities which are the ontogenetic product of totipotent precursors and which are delimited such that they are self-sufficient and can possess heritable phenotypic differences affecting lifetime reproductive success. These conditions are independent of the portion of the

		Ontogenetic axis	
		Single-celled precursor	Multi-celled precursor
Genetic axis	Meiotic cell-lineage	zygote	n/a
	Mitotic cell-lineage	stem cell	runners, buds, gemmules

Fig. 1. The combination of the ontogenetic and genetic views of individuality into a 2 × 2 matrix.

Box 1. Weismann's doctrine

Much contemporary evolutionary thought is still cast in terms of the soma/germ plasm doctrine developed by Weismann⁹, despite the fact that it has long been known that for at least 19 animal phyla, and for all plants and fungi, the basic premises of this doctrine are violated^{10,11}. The weismannian doctrine emphasizes (1) that ontogeny proceeds according to a fairly rigid, predetermined ontogenetic programme, (2) that germ line cells are sequestered early during ontogeny, and (3) that, as a consequence, a clear distinction between somatic (mitotic) and germline (meiotic) cell-lineages is warranted. In particular, the doctrine has focussed the attention on individual organisms which are the ontogenetic product of a zygote. This doctrine has deeply permeated several branches of biology, and has proliferated at least two widely held views regarding which kinds of entities count as individuals in an evolutionary context.

matrix (Fig. 1) in which the organism falls. In particular, such entities include many 'individuals' which have been widely ignored by inadvertent concentration on the upper left element of the matrix, for example ramets in clonal plants, or mitotically derived animals such as clonal aphids or cladocerans (Fig. 2).

Evolution in mitotic cell-lineages

Evolution within mitotically-derived individuals

Mosaicism – the prevalence of genetically different mitotic cell-lineages within an organism – is common and well-documented in all major taxa (reviews in Refs 1,11). Such cell lineages may contribute to evolutionary change, in the absence of meiosis, by competition between them. A mutant cell in a chimaeric parent has three possible fates. It can go extinct, it can go to fixation in a meristem and produce a genetically homogeneous new entity, or it can persist at levels between extinction and fixation. The first fate has no evolutionary consequences, but the other two do. Several theoretical studies^{8,13–15} address the evolutionary significance of such 'diplontic selection', both from the point of view of ridding the meristems of deleterious

mutations, and from the point of view of advantageous mutations going to fixation.

The asymptotic probability of a genetically homogeneous meristem arising can be surprisingly high (0.01–0.5, or even higher, depending on details of ontogeny), and the time required to reach the asymptotic fixation probability can be in the order of 50 mitotic cell cycles. Hence, the likelihood of a lineage of ramets giving rise to a genetically homogeneous individual is clearly high enough, and the time-scale is clearly short enough, potentially to contribute to evolutionary change (see also Ref. 1).

When mutant cell lineages generate genetically distinct and homogeneous new entities (through stochastic subsampling or differential proliferation), the new bodies are the developmental product of a single ancestral cell, and are physically and functionally separate. Hence, evolution in multicelled meristems is not qualitatively (only quantitatively) different from evolution in single-celled mitotic lineages: in both cases evolution ensues from a single cell without meiosis. In terms of Fig. 2, this amounts to evolution occurring solely by iterating right-hand loops (dashed arrows) without any involvement of the left-hand loop (solid arrows). The new entities interact, and have a life-history encompassing reproduction and death. They are formed by reproduction, not growth, and their genomes, if not disrupted greatly by genetic recombination, are inherited with very high fidelity¹².

The other possible fate of a mutant cell which has evolutionary significance is to persist at frequencies between zero and one. Such intermediate penetrance of a mutant initial cell is to be expected where the mutation rate is high with respect to the cell cycle time and the number of cells in the meristem, and the selection pressure is low, or when frequency dependent selection leads to an ESS comprising coexistence of mutant and wildtype cells. Under these conditions, a lineage *m1* may be replaced by a new mutant *m2*, and *m2* by *m3*, etc. without any lineage ever being fixed

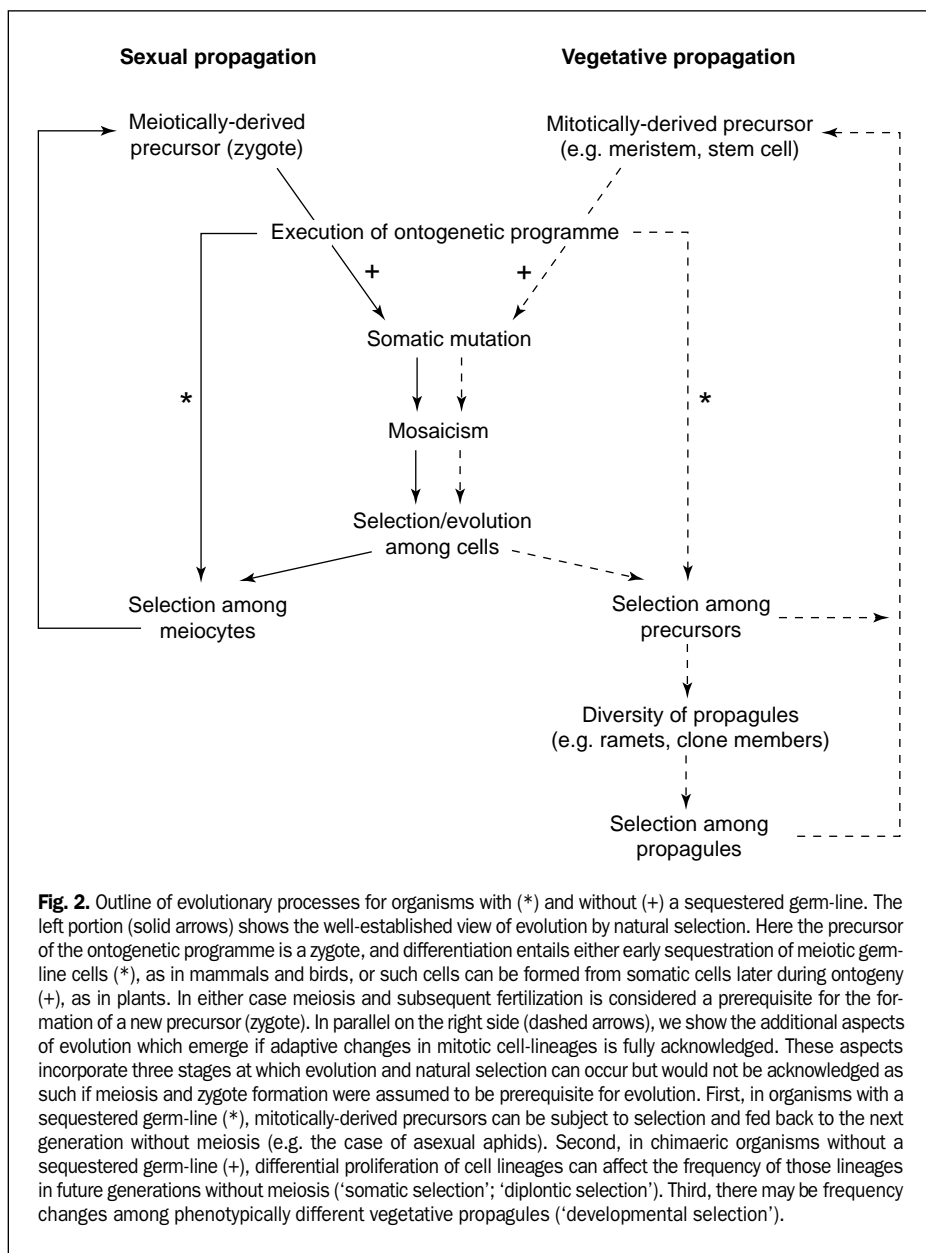
in the meristem. Thus we see that evolution may occur without genetic homogenization of lineages within meristems or their equivalents.

Evolution among mitotically derived individuals

When a mutant cell lineage has phenotypic consequences for some trait expressed in the ramet, there is opportunity for selection among ramets. Such selection in plants has been thoroughly analysed from a theoretical perspective¹⁶ but has rarely been studied empirically, the only case we know of being the papers by Breesse and co-workers^{17,18}. They contrasted predominantly asexual with sexual populations and demonstrated that somatic variation occurs within clones of *Lolium perenne*, that the level of somatic variation is related to the genotype, environment, and age of the clone, and that natural selection can operate on this variability. A major cause for the lack of examples of these phenomena in the wild is probably a lack of genetic data and breeding studies. The literature concerning propagation of naturally-occurring somatic mutations in crop plants abounds with examples of mutant entities of radically different phenotypes, which could easily have spread under natural selection¹⁹. Similarly, data on genetically modified organisms provide examples where small genetic changes, of the type which could occur by somatic mutation, persist or spread through populations²⁰.

In animals, there is a considerable body of theory and data that mitotically-produced entities can evolve at least as fast as meiotic ones, and may be long-lived on an evolutionary timescale^{21–23}. Genetic variation in clonal organisms has sometimes been demonstrated to arise from mutation rather than recombination and may have phenotypic consequences, e.g. mitotically-derived aphids having new traits^{24,25}.

Sitobion miscanthi is an aphid that reproduces by parthenogenetic apomixis (progeny derived from a stem cell by mitosis) in Australia²⁵. One lineage of these aphids has diverged by mutation and chromosomal rearrangement alone, from a successful recent colonizer. This divergence has produced at least three closely-related chromosomal races, one of which has known sub-lineages. The forms can exist sympatrically. Laboratory and outdoor experimental data show that these races (and even sublineages within races) have significant differences in their responses to temperature, and in the suitability of certain host plants. Under natural conditions there is measurable spatiotemporal variation in abundance of the forms, and differences in host-use. These data suggest that



divergence by mutation from a common ancestor has generated variation visible to natural selection in the wild.

Consequences for darwinian concepts

The life-cycle of a clonal plant can be seen as a meristem–meristem cycle, rather than as a zygote–zygote cycle²⁶. The meristem is the precursor of a ramet, whereas the zygote is the precursor of a genet which is split up into physically disconnected ramets. Hence, the virtue of the meristem approach is that a ramet (but not a genet) is a demographically, physiologically and ecologically distinct unit which squares with all those well established experimental protocols and conceptual issues that prevail in ecological/evolutionary research.

A ramet can give rise to new ramets via two alternative ontogenetic pathways, (1) by vegetatively budding off new ramets, or (2) by producing flowers which then

after fertilization and seed production give rise to new ramets developed by the emerging seedlings. By the former pathway the daughter ramets are mitotically derived, by the latter they are meiotically derived, but in either case genetic information is propagated between mother and daughter ramets, albeit this propagation is less certain by the second pathway (the probability of a given gene making it through meiosis is usually considerably lower than the corresponding probability pertaining to mitosis).

A ramet is thus perceived as the basic unit that is iterated during the life-cycle of a clonal plant, suggesting that the combination of pathways (1) and (2) which gives the maximum rate of increase in the gene(s) responsible for determining pathways will be the life-history molded by evolution (Box 2). In terms of the arguments of this article, this view thus acknowledges that mitotically as well as meiotically de-

Box 2. Evolution within a lineage of ramets

Let p and $(1-p)$ denote the probabilities of a ramet producing new ramets vegetatively and sexually, respectively. Then

$$df/dt = pm_v + q(1-p)m_s \quad (1)$$

is the rate of increase of a gene coding for p . m_v and m_s are the fecundities ensuing from the vegetative and sexual reproductive pathways, and q is the probability of an allele making it through meiosis. Thus, p is thought of as a phenotypic trait which defines a continuum of possible life-histories, and we should expect that for a given ecological context there is a life-history p_{opt} which gives a higher rate of increase of a gene coding for this p -value than of a gene coding for any other value. The rate of change of an active replicator (*sensu* Ref. 4), i.e. a genetic element which has a causal influence on its own probability of being propagated, is thus suggested as a meaningful measure of darwinian fitness.

rived progeny are new entities which may contribute to evolution by natural selection, so that both types contribute directly to darwinian fitness. Moreover, eqn (1) in Box 2 can be used as a conceptual framework for incorporating any type of mitotic evolution within ramets; for example, the first term in the right-hand side can be weighted by appropriate probabilities for functional persistence of a mutant initial cell in a meristem^{8,27}. Clearly, therefore, it lends itself to all types of mixed meiotic/mitotic reproduction.

Conclusions

There is a rich and ramified literature that is primarily concerned with the evolution of sex and the operation of 'Muller's ratchet' (recent reviews in Refs 28–30), i.e. it addresses the problem of why, undeniably, most multicellular organisms have included meiosis and zygote formation in their life cycles. Notwithstanding the relevance of that research for understanding why vegetative reproduction is usually a facultative, rather than obligate, trait, our main focus here is on the other side of the coin. That is, given the equally undeniable fact that many organisms have included mitotically-derived progeny in their life cycles, what, then, is the potential evolutionary significance of this mode of reproduction. In particular, we highlight that weismannian thinking is still hampering progress in this field.

A major incentive for abandoning the weismannian doctrine is that it has serious consequences when it comes to defining such basic concepts as 'fitness', or 'residual reproductive value' (RRV) for organisms not belonging to the upper left element of our matrix (Fig. 1). The traditional individual, the ontogenetic product of a zygote, is potentially immortal and therefore has potentially infinite RRV in

organisms such as *Lemna* or mitotically-derived aphids. No useful application of life-history theory is feasible for such organisms, nor is the celebrated concept of a genet (implicitly assumed to remain genetically unchanged) particularly useful in the context of the increasing evidence that mitotic cell-lineages can, and do, evolve.

Evolution of mitotic lineages appears the more likely in the light of the growing insights into genome function, including its hierarchical organization (gene regulators may control substantial regions of the genome), and the operation of mobile elements (e.g. transposable elements³¹). In this context, it is relevant to note that there is increasing evidence that different cell lineages within a ramet or its equivalent communicate cooperatively or competitively^{15,32}. It might be thought that potential meristem homogeneity could be limited by developmental factors. On the contrary, for many organisms, it seems that mutants can be propagated through the meristem. A variety of mechanisms exist by which cell lines can pass from one totipotent tissue into another¹⁹, and recent research has shown that the fate of individual cells in meristems can be much more labile than previously thought³³. In plants, there can be extreme non-linear phenotypic responses to small changes in genes and their expression, and shifts in states which occur in a meristem may be stably transmitted to future derivatives³⁴.

In terms of the ontogenetic argument, i.e. that the life cycle has to be contracted to a one-cell stage, we suggest that this idea derives from a confusion of two separate lines of thought. One is the idea (which we subscribe to) that the precursor of an individual must be totipotent. The other is the incidental fact that in most favoured model organisms (such as mammals and birds), such totipotency prevails only early in the blastula stage.

If these two lines of thought are decoupled, then a multi-cellular precursor – such as a meristem – is found to be equally acceptable as a vehicle for evolutionary change under natural selection as is a single-celled one. To be more precise, the difference between them is quantitative not qualitative. This is clearly borne out in the theoretical work reviewed above, and it is also suggested from the empirical evidence – albeit sparse – that adaptive radiation in mitotic cell-lineages can occur in organisms having single-celled as well as multi-celled precursors. The ambivalence that prevails in the literature concerning the need for a single-celled precursor ('single-celled or small'³; 'narrow bottleneck of cells'⁴) can be resolved. There is no need for a narrow bottleneck; all that matters qualitatively is that the bottleneck is totipotent.

In general, much evidence concerning adaptive evolution in lineages of mitotically-derived progeny is probably hidden in the literature simply because data that are in fact suggestive of adaptive evolution have been interpreted otherwise. Genetic variation among clones should conform to Hardy–Weinberg expectations³⁵ to provide evidence for undetected sexual reproduction, i.e. polyphyletic origins of clones, and similarly for animals². Otherwise such variation may, in fact, be due to adaptive evolution among lineages of ramets or their equivalents being subjected to different selective regimes.

Acknowledgements

We thank M. Gillings, P. Lundberg, T. von Schantz, J. Tuomi, M. Waycott, M. Westoby and three reviewers for useful criticism. TF acknowledges support for a sabbatical stay at Macquarie University from Lund University, The Royal Swedish Academy of Agriculture and Forestry, and The Royal Physiographical Society of Lund. This is publication 227 from the Key Centre for Biodiversity and Bioresources at Macquarie University.

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