Evolution via somatic genetic variation in modular species

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Highlights

Recent genomic data reveal that somatic genetic variation (SoGV) is widespread, but evolutionary consequences of this within-organismal level of genetic diversity are largely ignored.

In modular plant, animal and fungi species featuring somatic asexual (=clonal) reproduction, long life-span along with segregation of somatic variation into independent modules (ramets) may create phenotypic diversity as target for selection.

Recent genomic data suggest that SoGV can be transferred into gametes in species with late-sequestered, transient germlines (all plants and fungi, some basal animals).

Somatic evolution is nested within sexual reproduction and needs to be better integrated into population genetic theory for a large number of species encompassing plants, fungi and basal animals.

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Abstract

Somatic genetic variation (SoGV) may play a consequential yet underappreciated role in long-lived, modular species among plants, animals and fungi. Recent genomic data identified two levels of genetic heterogeneity, between cell lines and between modules that are subject to multi-level selection. Because SoGV can transfer into gametes when germlines are sequestered late in ontogeny (plants, algae and fungi, some basal animals), sexual and asexual processes provide interdependent routes of mutational input and so impact the accumulation of genetic load and molecular evolution rates of the integrated asexual/sexual life cycle. Avenues for future research include possible fitness effects of SoGV, the identification and implications of multi-level selection, and modeling of asexual selective sweeps using approaches from tumor evolution.

Somatic mutations and within-individual genetic heterogeneity

In multicellular species, **somatic genetic variation** (hereafter SoGV, see **Glossary**) is continually emerging from **somatic mutations** during mitotic growth and tissue regeneration, often at a higher rate than meiotic ones [1, 2]. As a result, all organisms are **genetic mosaics**, composed of cell lines with different genotypes [3]. Such somatic genetic variation accumulates with organismal age [4] that can be 100s of years in many clonally reproducing species [5]. While earlier theoretical and conceptual work has repeatedly suggested that somatically generated variation plays an important role in modular species (e.g. [6-11]), somatic genetic variation is still being treated as evolutionary noise or a dead end. Such a view likely arises because in species with an early differentiation of somatic cells from germline cells, any somatic genetic variation cannot be transferred to future generations. The underlying germline barrier concept [12] certainly applies to many unitary animal species but does not hold for the full diversity of multicellular life [13, 14].

Here, we focus on species with agametic formation of asexual offspring, often termed **clonal reproduction**, that has single or multiple cells of somatic tissue as precursors [15]. These species proliferate by producing new **modules** through diverse mechanisms such as budding, branching or fission (Figure 1), creating a population of **ramets** (a module existing as an independent physiological entity) belonging to a **genet** that dates back to a single zygote. While it has been acknowledged that this large portion of biological diversity residing in **clonal species** requires its own evolutionary analysis [5], many evolutionary textbooks still treat clonal reproduction via somatically produced precursor cells as an evolutionary oddity.

The genomic revolution has yielded a number of relevant data sets that directly address the importance of genetic heterogeneity within organisms at the full genome scale [3, 16-20]. Hence, it is timely to revisit some of the earlier thinking [6, 13, 14, 21] in order to expand the existing population genetic concepts by explicitly acknowledging the interplay between somatically and meiotically generated genetic variation, the distinct levels of selection within and among modules and the potential role of SoGV in adaptation.

Our perspective is motivated by (i) recent reports of substantial somatic genetic variation that agree with earlier theoretical predictions (ii) the discovery of mosaic variation that is rapidly segregating among modules of clonal species (iii) direct evidence for the transfer of somatic genetic variation to gametes in animals, fungi and plants and (iv) recent empirical findings for

multi-level selection within cell lines and among ramets. We also discuss whether SoGV can contribute to evolutionary adaptation and suggest research approaches that will better integrate evolutionary dynamics under asexual modularity with current standard evolutionary genetic models.

Abundant somatic genetic variation in multicellular species

As predicted from theory, (e.g. [7, 22], substantial somatic genetic variation has now been identified in clonal plants [3, 16], fungi [19], algae [20] and basal invertebrates such as corals [17, 18, 23]. Some species of fungi and of plants feature much less somatic genetic variation than anticipated, suggesting dedicated mechanisms of keeping somatic mutation rates low and hence, maintaining genome integrity [24, 25]. Currently, there is too little data to make any generalization with respect to abundance, genomic location of, and mutation rates generating SoGV, but it is likely that most of the detectable mutations are neutral or nearly so, while few are adaptive in line with the neutral theory of molecular evolution [26], and the existing data support this notion [16, 18]. SoGV either exist as **genetic mosaics** only carried by a subset of the cell populations of an organism, or as fixed variation carried by all cells of a given module or ramet, although currently only few data sets are able to distinguish among both types [16].

The detection of such mosaic SoGV is challenging as the power to detect low frequency variants that are present in, say 10% of all cells under diploidy requires substantial coverage to distinguish signal from technical error [27]. This issue is alleviated if SoGV are only quantified when fixed, a methodological restriction applying to most studies to date (but see ref [16]. This implies that derived somatic mutation rates may be underestimated (but see ref [25]), as abundant SoGV may remain undetected as mosaic genetic variation, and this in turns depends on mechanisms of module formation (see below “Segregation and fate of SoGV”).

Alternatively, single cell genomics has the potential to address SoGV in its mosaic state, specifically the mutational state of single somatic cells without the technical issue of detecting them as low frequency variants diluted among many other ancestral genotypes at target loci [2, 28] (Figure 2). Although today single cell genomics is still prohibitively expensive for larger surveys we can foresee that with a continuous drop in sequencing costs, analyzing many subsamples within ramets will soon be feasible. This will lead to a full, tissue specific characterization of the SoGV landscape in large genets.

Segregation and fate of SOGV depend on species life-history and reproductive mode

Once emerging as somatic mutations, the evolutionary fate of SoGVs has received very different attention depending on the study system. In **unitary** (=non-modular) species, positive selection on SoGV may initiate defective cell lines that lead to tumor growth, which has sparked the field of cancer evolutionary genomics [29]. Indeed, such defects often constitute a literal dead end of the affected organism and of all associated cell lineages carrying the causal SoGV. Approaches currently being developed in cancer evolutionary genomics may be useful for detecting asexual selective sweeps in modular organisms (Box 1).

Under clonal reproduction, the dynamics of SoGV change dramatically because module death is not equivalent to organism (or more specifically, genet) death. This implies that both, positive selection at the cell lineage level (causing cancer), and negative selection on the ramet level can operate without killing the entire organism, i.e., the genet. Second, the indeterminate (open) developmental program of such species may result in millions of ramets over time, creating a large asexual population of modules that is able to explore a considerable portion of the mutational space [7, 30].

Prerequisite for selection to operate at the module level is that **somatic genetic drift** moves SoGV from the initial mosaic status to fixation within modules (Figure 2). This type of drift has recently been proposed to segregate somatic variation among newly formed modules. The key idea is that only a subset of somatic cells is recruited to form the new module, i.e. they inevitably have to pass a genetic bottleneck [16], a process completely analogous to a genetic bottleneck and concomitant drift at the level of (a)sexual populations of unitary organisms.

How somatic genetic drift affects the fate of SoGV will depend on the morphological details of ramet formation, i.e. if new modules are formed via fission, fragmentation, budding or stolons (Figure 1). While the vegetative growth dynamics in vascular plants by means of apical meristems are well described [10, 31], less is known about the precise nature of module formation across the animal kingdom [32]. In the extreme, if only one cell is recruited as precursor for a new module, somatic genetic drift will progress the fastest. This seems to be the case in *Hydra* [33] and in fungal mycelial cells where any one cell can give rise to fruiting bodies [34],. In ferns, the drift process can also happen during growth of a single module since only a single cell in the apical meristem is responsible for new tissue formation [10]. Any mutation occurring in that cell immediately propagates through the entire plant module. However, this mode of vegetative propagation precludes the potential of within-ramet selection. On the other hand, (nearly) symmetric fission, as for example in sea anemones [35] or sea cucumbers [36] (Figure 1) comprises large cell populations, incurs the smallest bottleneck in cell population size and hence, will reduce the rate of somatic genetic drift compared to other modes of new module formation. The same should apply to fragmentation, e.g. as found in corals, where branches containing many polyps break off the colony (see also brown algae). No comparative study exists to date that quantifies somatic genetic drift as a function of the bottleneck size during and frequency of ramet formation.

Transfer of somatic genetic variation to the sexual cycle

In plants, the lack of an early segregating **germline** has long been acknowledged [10, 21, 37], even although direct evidence that somatically generated variation can be passed onto gametes /seeds is recent [3, 38]. Indeed, the evolutionary implications of accumulating SoGV throughout cycles of clonal reproduction remain understudied given that 40% of all plant families feature this type of reproduction [39]. In some species, mutational load is controlled somewhat by slower divisions in axillary meristems [3, 38], but the generality of these findings is unclear. Fungi do not segregate a germline at all [34], having apparently taken an alternate route to decreasing mutational load [24, 40].

In the animal world, the observation of a dedicated cell population responsible for gamete formation set aside early in ontogeny (Weismann's germline barrier [12]) has dominated concepts of evolutionary dynamics. While many animal phyla feature a strict and early

germline /soma distinction, including model species such as *C. elegans*, *Drosophila* spp. and all vertebrate models [41], pre-bilatarians may specify germlines later in development [42, 43] providing opportunities for the transfer of somatic mutations to the germline (Figure 3A). Recent evidence from corals shows that somatic genetic variation is indeed passed on to offspring via at least one round of meiosis [18]. This might happen via the processes of re-differentiation or trans-differentiation of soma cells into germline cells (Figure 3A), or the life-long, continuous differentiation of stem cells into soma and germline cells as observed in hydrozoans [23, 43, 44] and sponges [42]. Transfer to the germline implies that somatic mutations are recombined during meiosis [18] and thus can quickly be placed in many novel genetic backgrounds (Figure 3A), reducing the waiting time until any linkage between beneficial and detrimental mutations decays [45].

Given the diversity in developmental programs in modular organisms and the importance of timing between the arrival of a somatic mutation and its possible transfer through a meiotic event to the next generation, research into germline differentiation is critical. Among animal species, several stem cell lines may compete for dominance in giving rise to modules (Figure 3B) [46]. The competition dynamics would depend on whether modules are mosaics or homogenous, arising from just one stem cell line [47, 48]. Recent advances in cell sorting combined with single-cell RNA experiments to identify cell types provide an exciting avenue for rapid progress in this area for basal metazoa [49].

Multi-level selection and evolutionary adaptation

Mutation, selection and drift operate at two additional levels of biological organization in modular species, among cell lineages and among ramets [11] (Figure 3B), along with the "classical" selection on genotypes arising among sexually emerging genets. While recently cell lineage dynamics in induced pluripotent cells have also been observed in humans [50], the selection potential at the cell lineage level is much reduced owing to the low number of cell divisions (**cell depth**) during one gamete-to-gamete cycle in species with early segregated germline. In contrast, cell depth increases rapidly with organismal age among the proliferating tissues of continually forming modules under clonal reproduction [7, 30]. As of now, we are not aware of any cell depth study throughout cycles of agametic reproduction in clonal species, nor of the environmental conditions promoting high cell depth.

Modular, clonally reproducing species vary with respect to their sexual reproduction frequencies. At the extreme, sexual reproduction has been lost altogether [51], forcing such species to rely exclusively on somatic evolutionary processes including ramet-level and cell lineage-level evolution. Other species retain regular, if infrequent sexual reproduction resulting in occasional selection on recombined genotypes. To identify the driving forces and interactions among those three levels of evolutionary dynamics, variation in the degree of clonality among populations of the same species such as seagrasses or corals would be particularly instructive for future studies.

Whether or not selection dynamics among cell lineages and among ramets are synergistic or antagonistic depends upon the specific fitness effects of the arising mutations, and clearly needs more empirical research. An obvious example for antagonistic selection would be positive cell lineage selection, prominently featured by cancer tumors, that is also observed in ramets of modular species and would decrease fitness of the next higher level of selection. Negative selection against defective variants at the cell lineage level in some general

housekeeping metabolic pathways, on the other hand, should generally be in line with anticipated fitness effects at the ramet level, thus, act synergistically at both, cell population and ramet population level.

Multi-level selection may also speed up adaptive evolution by two processes. First, cell-lineage selection provides a first selection filter that reduces mutational load and delays Muller's ratchet within ramets [8, 52]. However, in diploid species, this requires that the genetic load is at least partially dominant. At the same time, this enriches for positively selected variants that can be subject to among-ramet level selection [31, 53]. With high-throughput genomics tools are now readily available to test these predictions. Preliminary evidence from large plant genets have revealed that dN/dS-ratios vary within versus among ramets, suggesting that indeed, two different levels of selection are operating [16]. In the absence of genomic information, few experiments have addressed the heritability of and selection on variation among modules/ramets versus genets [54, 55]. While recent work on bryozoan colonies [55] found no heritability in module traits, future experiments should ideally couple genomic and phenotypic assessments of inter-module differentiation.

Evidence from plant invasions [56], agricultural breeding via asexual propagation [57], direct experimentation of hereditary variation within clonal lineages [54, 58, 59]; and the very interesting cases of transmissible cancer [60] suggest a substantial potential for asexual adaptation under multi-cellularity. Given that many clonal species are at the same time ecosystem foundation species [61-63], studying this alternative route of adaptive evolution is highly relevant to conservation and ecological genetics [30]. While epigenetic processes have been proposed as another mechanism to produce within-organism phenotypic variation [64, 65], we need to first understand any "hard-wired" genetic changes as a basis for subsequent assessments of further modulating processes (Box **Outstanding Questions**).

Concluding remarks

One legitimate critique as to the relevance of the adaptive processes suggested here is that consecutive favorable mutations have to occur sequentially, as it was thought that they cannot be re-combined among parental genotypes [66, 67]. Yet, the relative benefits of sexual over asexual reproduction become similar in large populations [45], and indeed asexual populations targeted here can be very large ($>10^6$ modules, encompassing $>10^{12}$ cells at a single site for a large plant, respectively coral genet). Under clonal growth and expansion, this also increases the likelihood that rare "jackpot" events occur in terms of co-occurring, positive mutations [68]. Population sizes of cells and ramets both determine somatic genetic mutational input and the relative strength of selection in clonal species but remain largely un-quantified, and thus present an urgent target for future research (see Box "Outstanding Questions"). Further, there are ways in which somatic mutations can enter the germ line and be recombined, at least in many plants, fungi and some pre-bilatarians.

Long phases of asexual reproduction will also have marked and as yet understudied consequences for meiosis and recombination. In diploid species, abundant deleterious mutations become expressed in haploid gametes and hence, effectively removed from the population gene pool [10, 53]. Moreover, when sequential advantageous mutations have accumulated, recombination will break-up co-adapted genes (positive epistasis), increasing the variance in offspring fitness. Likewise, reducing the mutational load through recombination /meiosis after long periods of asexual (mitotic) reproduction is predicted to

produce a much higher variance among offspring phenotypes than under frequent sexual reproduction, and may even lead to the loss of sexual reproduction [51].

Evolutionary biology has made tremendous progress in explaining the emergence and maintenance of sexual reproduction despite the two-fold costs of sex [67, 69]. Here, we addressed the flip side of the coin, namely, how do a large number of species cope with extended phases of asexual reproduction that, according to conventional wisdom, precludes the emergence of genetic and phenotypic diversity and hence, adaptive evolution? With empirical data increasingly confirming earlier conceptual work [6-11], it is now timely to suggest a paradigm shift that acknowledges the evolution of modular species at multiple levels. Cell lineages evolve within ramets, which in turn are forming asexual populations featuring a mix of mosaic and fixed SoGV. Both of these levels of variation and selection are, in turn, nested within sexually reproducing populations of genets that are corresponding to the "classical" level of individuality in population genetics of unitary species, leading to potentially complex pathways of adaptation that merit further study.

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Figures and Figure Captions

Figure 1. Modular species are widely distributed across the eukaryote tree of life and encompass multiple clades of multicellular algae, fungi, and diverse animal phyla. Because multicellularity has arisen multiple times, the evolution of an indeterminate, modular growth form also evolved multiple times independently. New modules (paler shading throughout panels) can be formed simultaneously (plants, corals, algae), or sequentially, as in sea cucumbers, Echinodermata) or flatworms (Platyhelminthes). These latter bilaterian animal species are not modular by design, but nevertheless produce new modules by agametic fission. Among the animals, cnidarians and sponges do not possess an early-sequestered germline.

Figure 2. Somatic genetic drift in an abstract modular organism undergoing branching or budding. Subsequent branching events constitute cell population bottlenecks that progressively fix novel variants emerging within a lineage of ramets, while others are lost. Bulk sequencing of proliferating tissue and its descendant tissue will produce variant allele frequencies at frequencies below fixation, i.e. $f<1$ in case of haploid genome, $f<0.5$ in case of diploidy. The smaller the number of cells recruited to form a new module, the faster the rate of somatic genetic drift. Sample vials of bulk sequencing (left) versus single cell sequencing (middle/right) illustrate expected contributions of tissues to variant allele frequencies.

Figure 3. Evolutionary consequences of somatic mutations when they enter the germline. A). In most animals, the Weisman barrier between soma and germline prevents transfer. However, germline determination occurs late in plants, fungi and some basal metazoans. In the hydrozoa, for example, stem cells differentiate into germ cells throughout the life of the colony. In others, trans-differentiation of soma into germ cells may occur. Thus, as somatic mutations accumulate, some may enter the germ line [18]. Once in the germline, somatic mutations are recombined into different genetic backgrounds during meiosis similar to germline mutations. This reduces linkage between potentially deleterious mutations which otherwise would lead to increasing genetic load. B) Multi-level selection may also speed up adaptive evolution by providing a first filter of negative selection at the level of cell populations. On the other hand, the success of adaptive SOGV depends on whether they occur in stem cells and the specifics of how new modules arise. Homogeneous modules each arising from single, mutated stem cells may compete with each other at the within-genet level and be subject to selection.

Figure I (inside Box 1). A) Neutral somatic mutation accumulation in a growing population of cells or modules. Novel mutations, by definition, arise in a single cell at some time t. If at that time t, the overall number of cells or modules is $N(t)$, this mutation will be present in a total fraction $f = 1/N(t)$ of the entire population. If all present and future mutations are neutral, on average all mutations will remain at their initial frequency f. B) A single bulk sample of a population of cells allows us to reconstruct the distribution of variant allele frequencies. In a neutral process, we expect two characteristic peaks. One peak at high frequency represents mutations present in the most recent common ancestor cell of the sample. Those mutations are found in all cells of the sample. A second low frequency peak represents mutations that arose during the growth of the population and are only present in a subset of cells. Importantly, theory predicts that under a neutral process, the right-hand tail of that distribution scales proportional to $1/f^2$, e.g. we expect more mutations at lower frequencies. C) If a mutation has a fitness advantage, over time it will sweep to a higher frequency f than would be expected under a neutral process. Importantly, all neutral mutations also present in that cells will hitchhike to higher frequencies as well. D) The hitchhiking of neutral mutations on the back of a single mutation under positive selection leads to over-representations of VAF at intermediate frequencies and can cause an additional third peak in the VAF spectrum. The position and height of the intermediate peak can inform on the strength and timing of the selection event.

Text Boxes

BOX 1 (including Figure I). **Harnessing cancer adaptation approaches to detect asexual selective sweeps**. A pertinent problem of adaptation in the absence of recombination is that few (one) causal mutation(s) will drag many other neutral variants to (near) fixation. Recent studies on the somatic evolution of cancer provide unique insight into the dynamics of somatic genetic variation in modular species, including approaches to identify types of selection. The quantification of variant cell populations in healthy and cancerous human tissues has progressed tremendously in recent years [70]. One major approach to identify ongoing asexual selective sweeps is the clustering of **variant allele frequencies** (VAF) from bulk sequencing data [71]. Cell proliferation in self-renewing, healthy tissues or growing tumor populations is a continuous source for new SOGV entering the population at lowest frequency $f = 1/N(t)$, with $N(t)$ being the population size at time t. If variants are neutral and expand by drift only, we expect fewer variants at higher frequencies (Fig I). The precise scaling follows a power law that depends on the demographic properties of the tissue. In healthy tissue with an approximately constant population size, low frequency variants scale with $1/f$; whereas in an exponentially growing tumor population, low frequency variants scale with $1/f^2$ [72, 73]. SOGV under positive selection disrupt these power laws, as later occurring variants become overrepresented at higher VAF, giving the impressions of additional clonal clusters within the site frequency spectrum [74]. The heights and frequencies of these VAF clusters contain information on the timing and strength of the asexual selective sweeps. One needs to be cautious, however, to correctly adjust for expected neutral low frequency variants to avoid misinterpretations [75]. In modular species, selection can happen at two different levels, among cell lineages, and among modules characterized by differentially fixed genetic variants (Figure 2 B). While previous work for detecting selection in cancer operated at the level of cell populations, we see no principal obstacles against applying such approaches to module dynamics (Fig. I). Other types of

selection tests, e.g. dN/dS ratio tests of molecular evolution may complement VAF spectrum analysis.

Glossary

Cell depth	number of mitotic cell divisions between zygote and gamete formation
Clone	nearly genetically identical asexual population of ramets originating by clonal reproduction from a single zygote. In cancer research, cell population sharing the same genotype
Clonal reproduction	in multicellular species, the asexual and agametic reproduction by precursor cells derived from somatic tissue
Clonal species	plants, fungi or animal species displaying vegetative (asexual) reproduction by precursor cells derived from somatic tissue via mitosis
Genet	asexual population of ramets tracing back to a single zygote
Genetic mosaic	organism that features cell lineages with different genotypes, originating from somatic mutation in a subset of cell lineages. An alternative origin would be genetic chimerism, which is not considered here
Module	iterative morphological unit produced by vegetative (=asexual) reproduction, in clonal species such modules can potentially or de facto constitute physiologically independent entities (cf. Figure 1), called -> ramets
Multi-level selection	levels of selection emerging in modular species among asexually proliferating cell populations (within ramets) and among ramets
Ramet	physiologically independent module in a clonally reproducing, modular species
Somatic genetic drift	process of segregation of somatic genetic variation among ramets, resulting from bottlenecks of cells initiating a new module
Somatic mutation	mutation happening in somatic tissue via mitosis during growth or tissue regeneration in multicellular species
Unitary species	species with complex and non-redundant organs, typically featuring a determinate life cycle, in contrast to ->modular species

Variant allele frequency (VAF)	frequency of variant (novel) allele, emerging as a result of somatic (= mitotic) mutation. Mutations start at a VAF of 1/#cells. Variant allele frequencies in bulk sequencing are <1 (or <0.5 under diploidy) when variant alleles are not yet fixed but occur in the genetic mosaic phase

Evolution via somatic genetic variation in modular species

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Box: Outstanding questions

Mutational input: What is the abundance of mosaic and fixed somatic genetic variation among multicellular life? Is there a systematic difference in mitotic mutation rate between clonal and unitary organisms? How does the mutational input via mitosis vs. meiosis compare among species as a function of genet longevity and frequency of clonal vs. sexual reproduction?

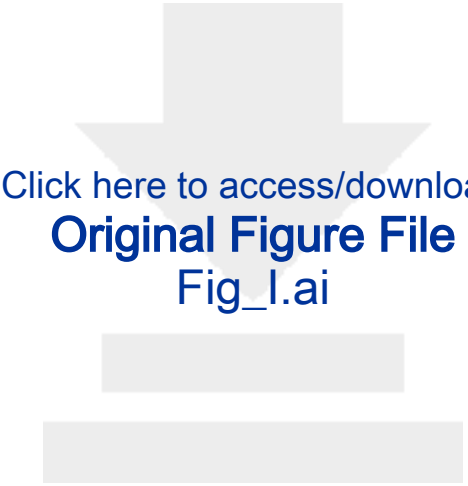
Somatic genetic drift: How does the population size of tissue initiating a new module determine magnitude of drift? Which and how many precursor cells are recruited for new modules? What are the implications of different ways to form ramets (i.e. fragmentation, fission, budding, branching) for the strength of somatic genetic drift? How does drift interact with selection?

Transfer of SoGV to the germline /sexual cycle: How can we differentiate between somatic and germline mutations if germ cells can be created continuously? Does this become an artificial distinction? Which part of genetic variation may end up in transient germlines? How can this be studied, e.g. using cell fate tracking?

Multi-level selection on SoGV: What is the evidence for multi-level selection? Is selection at the cell lineage level synergistic or antagonistic with respect to the ramet-level selection process? Is cell-lineage selection effective in purging deleterious mutational load and can this, in turn, explain the absence of senescence in many clonal species?

SoGV and evolutionary adaptation: Is multi-level selection promoting asexual adaptation? Does somatic genetic diversity provide an alternative route to adaptation in modular species? How can we effectively detect asexual selective sweeps given pervasive genomic linkage?

SoGV and speciation rates: are there systematic differences in speciation rate of unitary vs. clonal species? What is the relationship between the degree of clonal vs. sexual reproduction and the speciation rate?



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