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Opinion

Phenotypic plasticity through disposable genetic adaptation in ciliates

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Ciliates have an extraordinary genetic system in which each cell harbors two distinct kinds of nucleus, a transcriptionally active somatic nucleus and a quiescent germline nucleus. The latter undergoes classical, heritable genetic adaptation, while adaptation of the somatic nucleus is only short-term and thus disposable. The ecological and evolutionary relevance of this nuclear dimorphism have never been well formalized, which is surprising given the long history of using ciliates such as *Tetrahymena* and *Paramecium* as model organisms. We present a novel, alternative explanation for ciliate nuclear dimorphism which, we argue, should be considered an instrument of phenotypic plasticity by somatic selection on the level of the ciliate clone, as if it were a diffuse multicellular organism. This viewpoint helps to put some enigmatic aspects of ciliate biology into perspective and presents the diversity of ciliates as a large natural experiment that we can exploit to study phenotypic plasticity and organismality.

Highlights

Somatic selection, a form of phenotypic plasticity that was hitherto described only in multicellulars, is also present in ciliates.

Ciliates have evolved two genomes, one of which is a carrier of short-term disposable adaptations serving the long-term survival of the other.

The natural history of ciliates, and some of their genomic specificities, need to be reconsidered in the light of phenotypic plasticity by somatic selection.

The extraordinary genetic system of ciliates

Ciliates are a ubiquitous eukaryotic life form present in most aquatic ecosystems. Most ciliates (e.g., *Tetrahymena*, *Oxytricha*, and *Paramecium*) take a central place in food webs as planktonic predators of microorganisms [1]. Some species are (facultatively) sessile, commensal, or parasitic. Ciliates have a unique genetic system that is, despite their merits as model organisms, relatively poorly understood. They exhibit **nuclear dimorphism** (see Glossary): each cell harbors two different kinds of nuclei. The cell's phenotype is determined by the expression of a somatic nucleus (**macronucleus**). Meanwhile, the germline nucleus (**micronucleus**) is quiescent. Being facultatively sexual, periods of asexual reproduction, during which both nuclei divide independently, are alternated with sexual reproduction, during which meiotic products of the micronucleus are exchanged between mother cells (see Figure 2 in [2]). Upon fertilization, these gamete nuclei fuse to form a new nucleus, which divides and gives rise to both a new micronucleus and macronucleus. Next, the old macronucleus disappears.

Since a genetic change has a strikingly different fate depending on whether it occurs in the micronucleus or macronucleus, the two nuclei have distinct evolutionary dynamics. Figure 1 illustrates how, in ciliates, diversity is generated and selected from at the level of the macronuclei, which have an extraordinary capacity to generate somatic genetic diversity (see following text). Since the macronucleus determines the phenotype, it is under selection during periods of vegetative growth, adapting to its environment in the same way that the genome of any other asexual unicellular organism does. Meanwhile, the silent micronucleus independently accumulates mutations that become exposed to selection only upon sexual reproduction, when a new macronucleus is derived from the new micronucleus. This leads to the presence of two levels of selection: the individual cell with its phenotype (and hence its fitness) determined by its macronucleus, and

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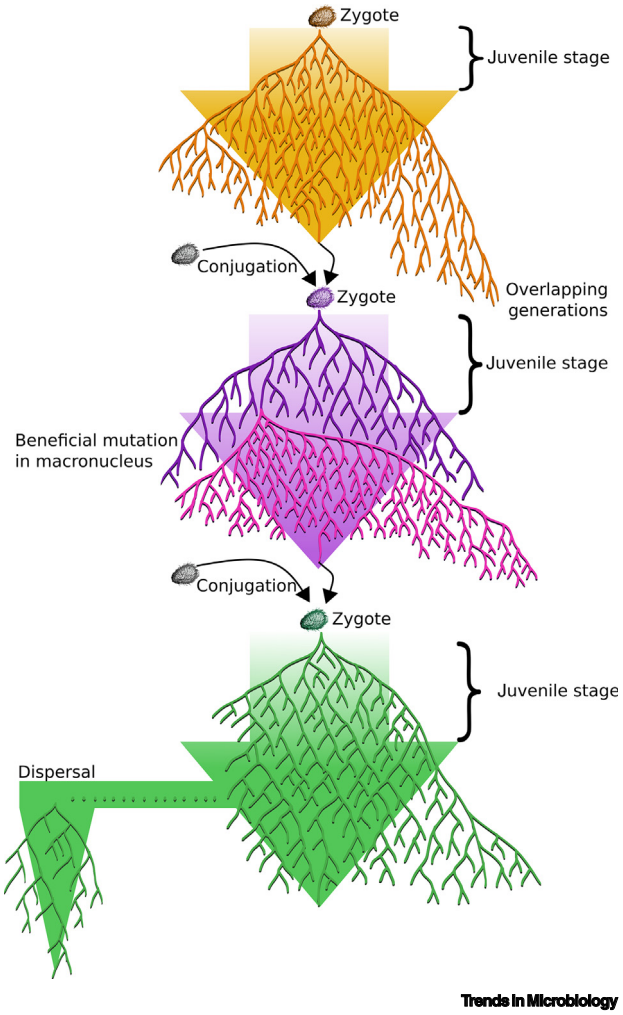

Figure 1. Schematic representation of three successive sexual generations in a typical ciliate Genealogies represent dividing macronuclei, while the large arrows represent the micronuclear genotype of the entire clone. Since not all cells reproduce sexually at the same time, and different clones may be simultaneously present, generations are overlapping. For illustration purposes, only one sexual event per clone is depicted here. In the second generation, a novelty on the level of the macronucleus confers a short-term fitness benefit (pink lineage), making faster-dividing cells outcompete other members of the clone that do not harbor the beneficial somatic variant. As is typically the case with phenotypic plasticity, this novelty is not passed on to the next generation. The third generation demonstrates that a clone can be spatially structured in several populations as a result of dispersal events.

the entire **clone** with its fitness determined by the ability of its micronucleus to generate well-adapted macronuclei.

The only nucleus that can ultimately contribute to the gene pool is the micronucleus, which therefore constitutes the genotype of the clone. We argue that, on an evolutionary level, the clone can be considered as the main unit of selection [3], and hence should be considered a **genet** (genetic [4] or evolutionary [5] individual). The ciliate cell can be thought of as a **ramet** (functional [4] or physiological [5] individual). In most ciliate species, cells separate after asexual division. The typical ciliate clone may thus be viewed as a diffuse multicellular organism (Box 1) consisting of physiologically independent somatic cells that share a germline.

Variable environments: phenotypic plasticity and bet-hedging

Here, we propose that the adaptation of the macronucleus constitutes a form of phenotypic plasticity: the ability of a single genotype to give rise to multiple phenotypes in different environments [6–8]. Phenotypic plasticity takes contrasting forms, including **passive phenotypic changes**, **generalized responses**, or **developmental switches** [9,10]. Examples of such sensing and

Glossary

Amitosis: division of a cell nucleus without the mitotic mechanisms that normally ensure the equal distribution of sister chromatids over daughter nuclei.
Bet-hedging: also known as adaptive coin-flipping. Evolutionary risk-spreading strategy that may evolve in the face of unpredictable environmental variations, where the temporal variance in offspring fitness is minimized at the expense of their arithmetic mean fitness.
Ciliates: a eukaryotic phylum, supported by several morphological synapomorphies, for example, somatic kinetids having a postciliary microtubular ribbon arising from triplet 9 and nuclear dimorphism. Common almost anywhere in water, with cell size varying from 10 micrometres to a few millimetres, mainly heterotrophic but also osmotrophic and parasitic.
Clone: ensemble of individuals (typically cells) that share recent common ancestry through asexual reproduction.
Conflict: situation in which two genetic entities (such as genomes) can each affect a joint phenotype, but they gain from moving it in opposite directions. Conflict can be ongoing, or it can be controlled, in which case we speak of potential conflict or conflict of interest [34].
Developmental switch: plasticity mechanism in which alternative developmental trajectories are chosen based on information from the environment.
Generalized response: an unspecific (physiological) plastic response that can lead to tolerance of a wide range of environmental conditions (for instance heat-shock protein expression [10]).
Genet: a genetic individual that arises from a single zygote. May consist of multiple physiologically independent units (ramets) that are formed through vegetative growth.
Genetic assimilation: a selective process by which an environmentally induced phenotype becomes constitutively produced.
Internal eliminated sequence: a stretch of DNA present in the micronuclear genome that is excised during macronucleus formation.
Macronucleus: the ciliate somatic nucleus. This nucleus is transcriptionally active and determines the majority of phenotypic traits. It is replaced after each sexual reproduction.

Box 1. The organismality of the ciliate clone

Whether a group of unicellulars can be considered an organism is a long-standing discussion. Ciliates have received little attention in this context, despite their unique genetic configuration that makes the clone a focal unit of selection, and hence, an evolutionary individual. However, not every evolutionary individual qualifies as an organism. While authors seem to agree that nested levels of biological individuality can coexist, criteria to define an organism are typically more restrictive [42,63,71–73]. Organismality is considered a derived state of a biological system resulting from feedback between natural selection and functional integration [71]. This culminates in the simultaneous concentration of evolutionary individuality (low conflict) and functional individuality (high cooperation) at one level of biological organization [42]. Though spatial contiguity is present in the majority of organisms, it is not considered a necessary condition (e.g., the eusocial insect colony) [42].

There is an important distinction between ciliates and other facultatively sexual organisms with hierarchical levels of selection. New genotypes that arise within a plant genet or a yeast clone can gain evolutionary traction and compete for their place in the gene pool. This heritable variation in fitness is a source of genetic conflict within the genet or clone [68,74]. In ciliates, however, somatic variation in fitness is not heritable beyond the next round of sexual reproduction due to the Weismann barrier. Therefore, individual cells do not represent a fully nested level of selection equivalent to the ramets of a plant. Much rather, the ciliate situation is reminiscent of a multicellular organism in which somatic cells benefit from the transmission of a copy of 'their' genome, which does not reside in their own nucleus (see Figure 2 in main text), or of a **superorganism** such as a eusocial insect colony, in which individual workers serve the transmission of the genetic material of their close kin.

Organismality is also defined in terms of functional integration [5,71], or cooperation between constituents of a biological entity [42]. Little is known about cooperation in ciliates, but it has been observed that *T. thermophila* cooperates under the form of cell aggregation to exchange growth-promoting macromolecules [75,76]. Decisions to aggregate provide fitness benefits during dispersal [77] and are influenced by the presence of kin [78]. Thus, even though the individual is divisible (see Figure 1 in main text), it is capable of self/nonself discrimination, which is a property that has been highlighted as a criterion for functional individuality [5,79].

Ciliates also show some degree of functional integration: depending on the age of the clone, cells are more prone to grow vegetatively or reproduce sexually. Indeed, a ciliate clone exhibits patterns of development and life-history traits that are comparable to those of a facultatively sexual multicellular with overlapping generations (see Figure 1 in main text). Each sexual generation starts off as a zygote that gives rise to a clone of vegetatively dividing cells. During the early stages of vegetative growth, processes reminiscent of embryonic development are observed in some species, with programmed macronuclear chromosome loss [80–82] and a sexually immature juvenile stage [83,84]. Senescence is also observed: after a number of asexual divisions, cells experience higher mortality and reduced fecundity, the only way to rejuvenate being sexual reproduction or autogamy. While senescence at the clone or genet level is extremely rare in clonal organisms [85], it has been observed in a wide variety of ciliates [86,87], with the notable exception of amicronucleated *Tetrahymena* lineages which, arguably, have lost the higher level of evolutionary individuality seen in other ciliates.

Many properties of ciliate biology plead in favor of viewing a ciliate clone as an organism with a shared genotype (the micronucleus), and with its generation time defined as the time between two rounds of sexual reproduction. This viewpoint is further corroborated by the fact that micronuclear mutation rates fall only within the range of known eukaryotic mutation rates per generation if we consider the sexual generation time [88,89]. The discussion about the extent of organismality of the ciliate clone needs further input from experiments and observations, especially about functional integration and cooperation. Given that organismality is a continuous [42,68,71] and context-dependent [63] quantity, it may well be that different ciliate species take a different place in this spectrum.

responding strategies in microorganisms include the lactose operon in *Escherichia coli* [11] or fruiting body formation from individual *Dictyostelium* cells upon starvation [12]. By modulating the direct link between genotype and phenotype, phenotypic plasticity is key to an organism's fitness [13]. When phenotypic plasticity provides a fitness benefit sufficiently high to select for its genetic basis, and when several environments are regularly encountered during evolutionary history, it can improve the match between phenotype and environment and is, therefore, adaptive [14].

However, cases exist in which reliable cues to predict the future state of the environment are unavailable. Such conditions are expected to favor diversified **bet-hedging** strategies, in which an individual increases the phenotypic variation among its offspring (be it sexual or asexual), resulting in high fitness of some offspring in a particular environment, while others may perform poorly. In this manner, the temporal variation in fitness of the genotype is buffered [15–17]. Various molecular mechanisms exist to constitutively generate phenotypic heterogeneity

Micronucleus: the ciliate germline nucleus. It is transcriptionally silent, divides by mitosis, and undergoes meiosis preceding sexual reproduction. A mitotic sister of the micronucleus gives rise to the new macronucleus after sexual reproduction.
Nuclear dimorphism: the characteristic of having two different kinds of nuclei in a cell.
Organismality: the ensemble of properties that make a biological entity function as an organism. Such properties include cooperation and communication among its constituents, physiological integration, recognition of self, and resolution of genetic conflict.
Passive phenotypic changes: any plastic changes not regulated by the organism.
Phenotypic assortment: the fixation of a haplotype (corresponding to a macronuclear chromosome) through multiple clonal divisions due to unequal chromosome segregation. Sometimes named, more adequately, 'allelic assortment'.
Ramet: a member of a genet with a high degree of structural and physiological independence.
Reaction norm: the pattern of phenotypic trait expression of a given genotype along an environmental gradient.
Somatic mosaicism: the presence of nonheritable genetic variation within one organism.
Somatic selection: amplification of beneficial phenotypes within a pool of massively produced somatic variants through feedback with the environment. Also called developmental or epigenetic selection [8].
Superorganism: a social unit composed of individuals from the same species, manifesting a high degree of collaboration through division of labor and the presence of a specialized reproductive cast. Conflict among its constituents is reduced due to high relatedness.
Transgenerational phenotypic plasticity (intergenerational phenotypic plasticity): plastic modification resulting from the environment experienced by any ancestor, commonly referred to as 'parental effect' when plasticity results from the environment experienced at the preceding generation.
Weismann barrier: originally described in multicellulars as a physical distinction between immortal germ cells

for a given genotype, allowing organisms to persist in fluctuating environments [18]. Well-characterized examples of bet-hedging include persisters in bacteria [19], and viral latency [20]. A bet-hedging strategy is not exclusive, and heterogeneity can be fine-tuned by phenotypic plasticity, such as in amphicarpic annual seeds [21].

destined to produce gametes, and disposable somatic cells making up the body.

Another strategy to deal with unpredictable environmental fluctuations is **somatic selection** [8,10]. This is a noncanonical form of phenotypic plasticity in which the phenotype–environment match relies on the production of a wide range of phenotypic states within one individual, out of which the best performing variants are selected through feedback between the environment and the developing individual [8,22]. Examples of somatic selection include the development of plant roots as a function of their ability to provide the plant with nutrients and water [22], or human learning, where, among trillions of synaptic connections, some are eliminated while others are reinforced upon their successful interaction with environmental cues [23]. Mechanistically, somatic selection does not look like a typical form of phenotypic plasticity with a canonical phenotype-to-environment mapping (**reaction norm**) shaped by evolutionary history. Conversely, in somatic selection, the feedback between environment and phenotype takes place within the lifetime of the individual [10].

Both in somatic selection and diversified bet-hedging, multiple phenotypic variants are generated from one genotype, but in bet-hedging, the distribution of initially generated variants is generally considered independent of the current environment (but see [21]), while in somatic selection, the distribution of phenotypic variants is modulated through constant feedback between phenotype and environment [10]. In addition, bet-hedging and plasticity by somatic selection differ on the level at which selection is acting. In somatic selection, variability is generated and selected from within an individual, while bet-hedging is based on interindividual phenotypic variation among offspring. The distinction is generally clear for organisms with classical life cycles. In plants, variation in seed dormancy is a form of diversified bet-hedging [17] while root proliferation or atrophy as a function of environmental conditions is a form of phenotypic plasticity by somatic selection [22]. Conversely, in strictly clonal microbes, the distinction may be dubious. Phenotypic heterogeneity within a clone can be considered diversified bet-hedging [16], but if members of a clone are joined together as a unit of selection it may also be seen as part of a somatic selection strategy.

Disposable genomes allow for somatic selection

To date, phenotypic plasticity by somatic selection has been conceptualized only for multicellular organisms: diversity is generated and selected from at the level of somatic tissues, while the presence of a physically distinct germline guarantees genetic continuity and a return to the naive state at every new generation. Since the ciliate clone is an evolutionary individual with its genotype contained in its germline micronuclei, genetic adaptation of its macronuclei is disposable and merely serves to generate the best vehicles to escort its genotype to the next sexual generation, as does the soma of multicellulars. We therefore argue that the disposable genetic adaptation of the ciliate macronucleus may be seen as an overlooked case of somatic selection, occurring at the level of a clone of unicellulars.

A comparison with the vertebrate adaptive immune system, a prime example of phenotypic plasticity by somatic selection, is illustrative (Figure 2). In the same way that the combination of all antigen-recognizing cells constitutes the immunological phenotype of a vertebrate individual, the total phenotype of the ciliate clone results from the ensemble of single-cell phenotypes that have been selected by the environment. The phenotypic plasticity of the clone resulting from somatic selection is distinct from, but complementary to, the more classical phenotypic plasticity at the level of the physiological individual (the cell).

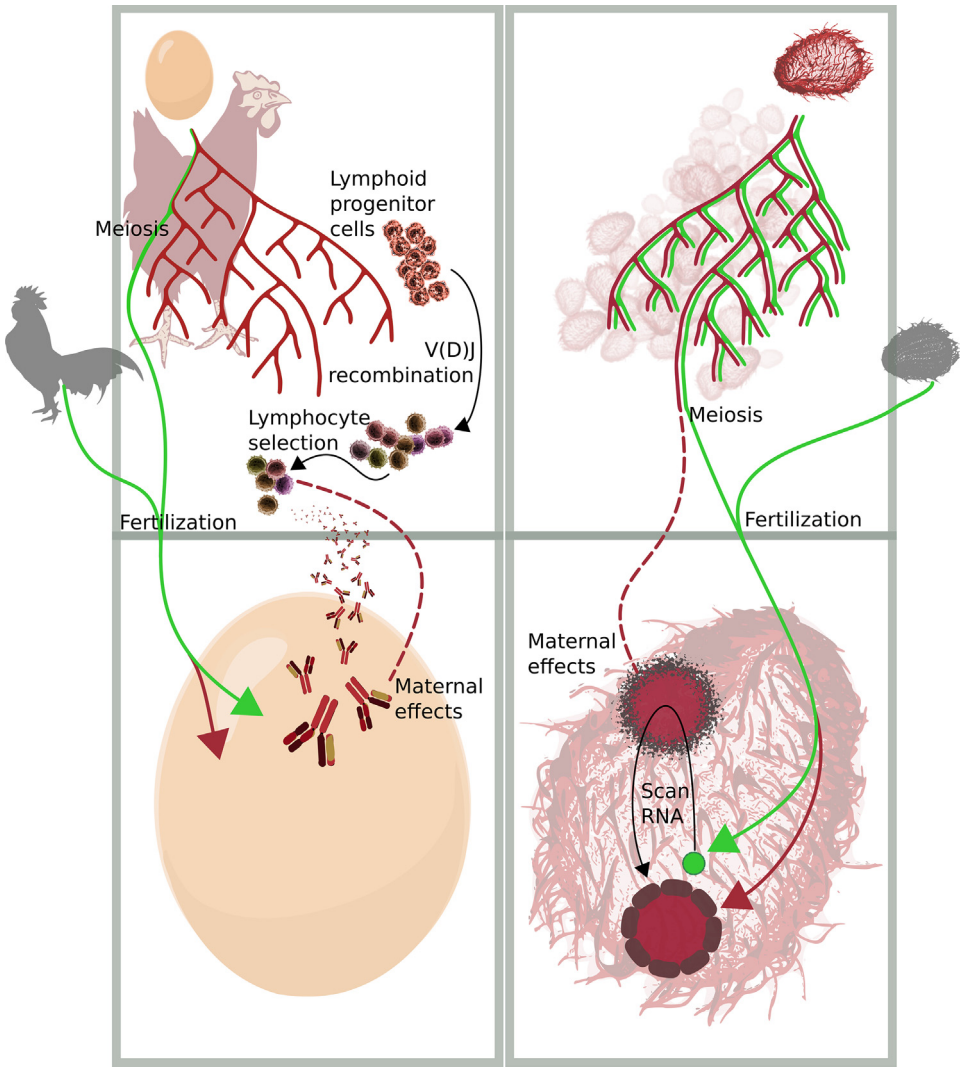

Figure 2. Comparison of phenotypic plasticity by somatic selection in a vertebrate and a ciliate. In both vertebrates (left-hand panel) and ciliates (right-hand panel), cell populations start off as a single zygote cell after fertilization. In vertebrates, the zygote gives rise to a multicellular individual with only the germline retaining its totipotency (green). The somatic cell lines (red) produce a variety of stem cells differentiating into all tissues that make up the body. One example of phenotypic plasticity by somatic selection in vertebrates is the acquired immune system. The cells giving rise to lymphoid progenitor cells undergo reshuffling of specific portions of their genome (immunoglobulin locus) through V(D)J recombination. This leads to a genetically heterogeneous population of B lymphocytes (**somatic mosaicism**) that receive proliferation signals depending on their match with the environment (antigen recognition). While this process generates genetic diversity at the level of the soma, the genotype of the individual remains unchanged, since it is sequestered in the germline. In ciliates, the cell population issued from the zygote is divisible, each cell carrying along a copy of the germline genome (green and red lines are never separated). Genetic heterogeneity is enhanced by amitosis (Figure 3), and somatic selection acts on each macronucleus independently. In both vertebrates and ciliates, the production and elimination of unfit cells are costly and the somatic genetic adaptations are disposable. However, some nongenetic aspects of the acquired traits can be transmitted to the offspring through maternal effects, for instance by passing on antibodies in egg yolk [69] or by the structuring of the new macronucleus through scan-RNA in ciliates [70].

The parallel with multicellulars (Box 1) is not absolute. In both cases, the expression of somatic genomes produces heterogeneous phenotypes, on which selection can operate depending on individual cell performance. The resulting repertoire of somatic genomes is associated with a

unique germline that evolves at the time scale of sexual generations. However, in ciliates, each somatic genome is associated with its own physical copy of the germline, while in multicellulars all somatic genomes are associated with a single, physically distinct germline. In a multicellular, selection acts more homogeneously on all physically coupled somatic genomes, whereas cells of a ciliate clone move independently and can disperse away from each other (Figure 1). As a result, somatic genomes of a ciliate clone can simultaneously be exposed to different selective pressures while silent mutations in their physically associated germline genomes hitchhike along.

Amitosis generates a huge somatic genomic variability

Phenotypic plasticity by somatic selection in ciliates is facilitated by several unusual properties of the macronucleus, directed towards the generation of somatic genetic heterogeneity. While the micronucleus is a typical diploid eukaryotic nucleus that undergoes mitosis during phases of asexual reproduction, the macronucleus is polyploid and divides **amitotically**. The exact process of macronucleus formation from the micronucleus template differs markedly between ciliate lineages but it always involves replication to a higher ploidy, fragmentation into smaller chromosomes, and sometimes extensive shuffling [2,24,25]. In *Tetrahymena thermophila*, the micronucleus has five diploid chromosomes, while the macronucleus has 181 chromosomes in about 45 copies each. In *Oxytricha trifallax*, macronuclear chromosomes are fragmented down more or less to the level of individual genes, giving rise to ~16 000 chromosomes occurring in copy numbers of up to several thousand each.

Along with centromeres, the macronucleus has lost its ability to form the mitotic spindle and metaphase plate, structures that normally guarantee the equal distribution of sister chromatids to the daughter nuclei. Therefore, the macronucleus divides amitotically, with a stochastic component to the partitioning of chromosomes between daughter nuclei, generating substantial somatic genetic diversity (Figure 3).

First, amitosis impacts the level of heterozygosity in the macronucleus. In a diploid multicellular, any locus that is heterozygous in the germline will be heterozygous in all somatic genomes, regardless of the number of divisions. Conversely, the partitioning of parental alleles during macronuclear division is a stochastic process. In any given macronuclear lineage, and in the absence of selection, each parental allele, as well as any *de novo* mutation, tends to either get lost or drift to fixation (Figure 3, proportion of color shades), a phenomenon known as **phenotypic assortment** [26]. In laboratory experiments, phenotypic assortment has long been used to obtain populations fully homozygous for a chromosome carrying a selective marker (e.g., antibiotic resistance). Fixation by neutral drift is expected to occur after ~200 divisions when ploidy is ~45 as in *T. thermophila* [26]. In species with higher ploidy, such as *Paramecium tetraurelia* (ploidy ~800) or *O. trifallax* (ploidy ~2000), fixation may occur much later, or may not happen within the lifetime of the clone. The fixation of an allele may be accelerated by directional selection, or it may be prevented by balancing selection. The result of phenotypic assortment is the production of a multitude of combinations of allele proportions at different loci, among the cells of a clone.

Second, amitosis has an impact on the ploidy of macronuclear chromosomes. While during amitosis, daughter macronuclei may inherit about equal total numbers of chromosomes, the partitioning of each individual chromosome may vary by chance [27,28]. This leads to stochastic copy number variations of individual macronuclear chromosomes among cells (Figure 3, height of bars), on which selection can act. The degree of stochasticity in this system may vary between species and is subject of debate. Some studies suggest that regulatory mechanisms prevent lethal ploidy variations [27,29], while others suggest that balancing selection is sufficient [28,30]. Partial ploidy changes are a quick and reversible way to alter patterns of gene expression. In

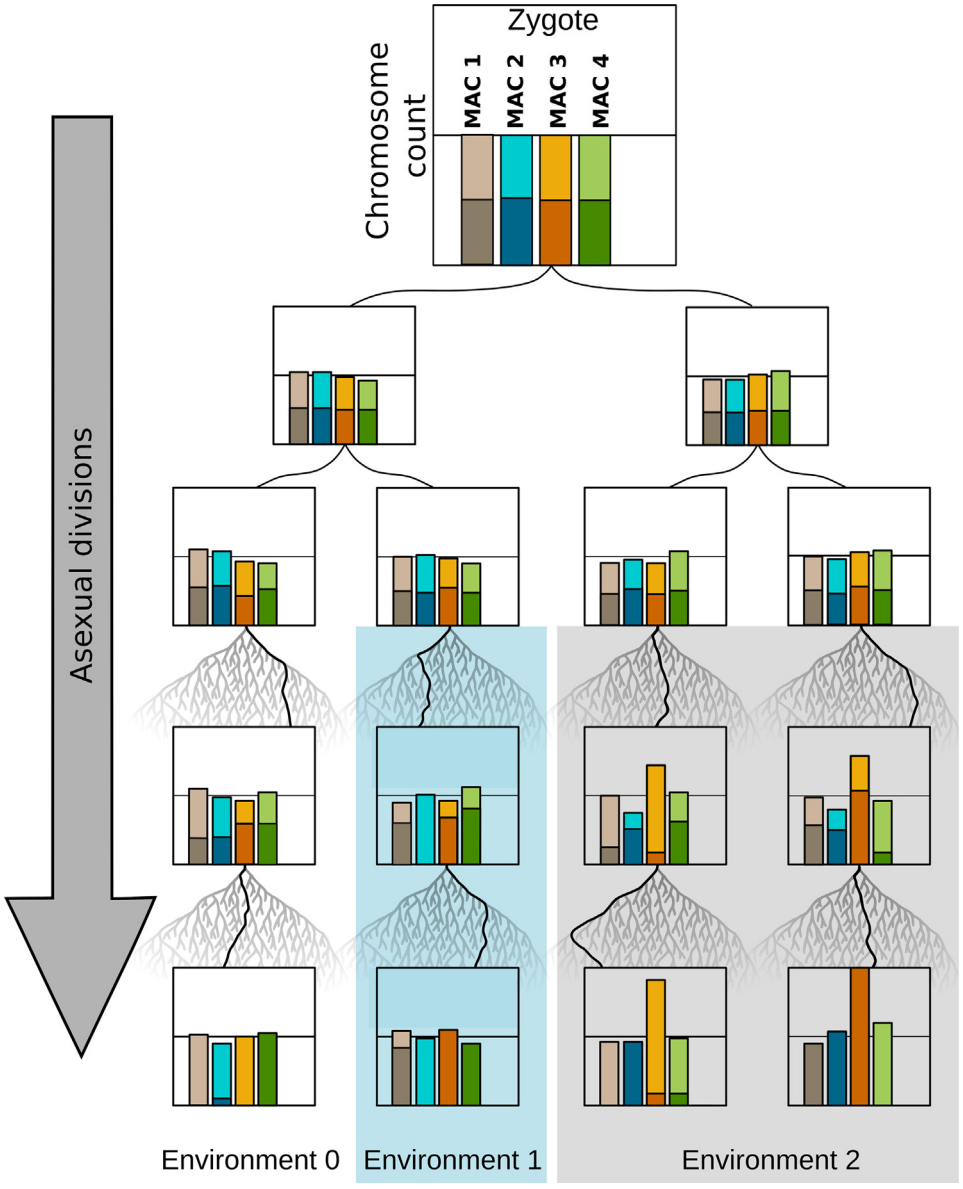

Figure 3. The generation of macronuclear diversity by amitosis. In this schematic representation of a ciliate clone, each square represents one cell. Each bar represents the counts of one macronuclear chromosome. Only four (out of many) macronuclear chromosomes are shown. The two color shades within each bar represent the two parental alleles. The zygote starts off with equal copy numbers of each chromosome, with a 50:50 distribution of parental alleles (equal proportions of the two color shades per bar). Since, during amitosis, chromosomes are 'drawn' from the pool of chromosomes, like marbles from a bag, deviations will appear both in numbers of chromosomes and in ratios of parental alleles. After one or two cell divisions, small deviations from equal partitioning of chromosome numbers start to appear (bars are above or below the median line). Then, only four lineages are shown after multiple rounds of asexual divisions (represented by genealogical trees). They grow under different conditions. In environment 0, which is the neutral scenario, frequencies of parental alleles deviate stochastically from equal distribution and at the end, in three out of four macronuclear chromosomes, a single parental allele has drifted to fixation (phenotypic assortment). In environment 1, the light-blue allele of chromosome 2 has a selective advantage, leading to the fixation of this allele faster than expected by neutral phenotypic assortment. In environment 2, there is a selective advantage for cells with higher copy numbers of macronuclear chromosome 3. Only four (out of many) macronuclear chromosomes are shown (MAC 1, 2, 3 and 4).

Paramecium bursaria copy-number variation of macronuclear chromosomes is low for chromosomes harboring housekeeping genes and high for those carrying environmental response genes [31]. Likewise, in the presence of toxic Cd^{2+}, the copy number of a chromosome harboring two metallothionein genes increased fivefold in *T. thermophila* [32]. These partial ploidy changes were reversible upon relief from heavy-metal stress, and could be reinduced upon a new exposure to Cd^{2+} in as little as a week, an interesting example of reversible phenotypic plasticity. While partial ploidy changes and phenotypic assortment certainly act in the wild, it remains to be evaluated to what extent they contribute to phenotypic plasticity by somatic selection.

Ciliate somatic selection and intergenomic conflict

Evolutionary **conflict** arises when multiple genetic entities, present within an individual, have divergent interests where they would benefit from moving the phenotype in opposite directions [33,34]. Conflict can occur between genetic entities within the same genome (intragenomic conflict [35]), between multiple genomes within an organism (intergenomic conflict, e.g., [36]), between soma and germline within a multicellular organism (cancer [37]), or even between reproductive and non-reproductive casts within a eusocial insect colony [38]. Multiple solutions appeared to reduce these costly conflicts, for instance, uniparental inheritance of mitochondria [39], cell-cycle checkpoints to prevent neoplastic growth [37], or policing worker-laid eggs in honeybees [38].

Within a ciliate clone, the presence of multiple levels of selection may lead to divergent interests between the micronucleus and the macronucleus [40,41]. From the perspective of the micronucleus, selection of the best macronuclear genomes within the clone provides the best odds for survival until the next sexual reproduction. However, since sex leads to an evolutionary dead-end for the macronucleus, the latter may gain a short-term benefit from the loss of the micronucleus, especially if such lineage has a growth advantage because it no longer has to replicate an extra nucleus. This tension sets the stage for intergenomic conflict that bears similarities with conflict between genomes of a cancer cell and the germline (selection on the somatic cell level [34,37,42]), or between nuclei in multinucleate ascomycete fungi due to within-mycelium selection [43]. The question is how this potential conflict is resolved in ciliates.

One extreme resolution is the complete 'victory' of the soma through the loss of the micronucleus, as seen in some *Tetrahymena* species [44]. Inevitably, such lineage also loses sexual recombination, and it is unclear whether the primary driver of micronucleus loss is the resolution of intergenomic conflict, or other dynamics related to the loss of sex. At a macroevolutionary level, the long-term survival of asexual lineages is generally limited to stable environments [45], and ciliates are no exception: although amicronucleated lineages in laboratory cultures have been known for a long time (e.g., [46]), in nature, they have been described only in the genus *Tetrahymena* [44].

Conversely, amacronucleated lineages have been observed only after experimental manipulation, and they are unstable. Nonetheless, the ability to divide the macronucleus was lost in the class Karyorelictea, in which a new macronucleus is generated from the micronucleus at each cell division [24,47]. Whether this loss evolved primarily as a resolution of genomic conflict, or rather as a loss of plasticity, needs further research. The long-term maintenance of phenotypic plasticity by somatic selection relies on the balance between the costs to produce numerous potentially unfit variants and the benefit of the fit between the selected phenotypes and the encountered environments [22]. Perhaps in the case of Karyorelictea, this cost–benefit balance has tilted because of the stability of their environment (sediment), or their slower pace of life (millimetric cells).

The loss of the micronucleus, and perhaps also the loss of amitosis, are extreme resolutions of intergenomic conflict. The primary solution that allows the long-term coexistence of the two

genomes lies in the presence of a **Weismann barrier**: a physical distinction between the soma and the germline. The Weismann barrier has been suggested to be an important mediator of conflict reduction in major evolutionary transitions because it curtails the evolutionary potential of the lower units of selection, taking away their evolutionary interest to behave selfishly [48–50]. In multicellular organisms, the Weismann barrier is achieved through the confinement of the germline genome in specific cells. In ciliates, germline and somatic genomes are in close proximity within the same cell, so specific mechanisms should have evolved to prevent DNA exchanges. For instance, nuclear membranes never disappear throughout the ciliate life cycle [51], providing a simple physical barrier between micronucleus and macronucleus. Also, massive RNA-guided rearrangements occurring during micronucleus-to-macronucleus transformation effectively reduce the similarity between the two genomes, making homologous recombination less likely. In *T. thermophila*, about one-third of the micronucleus is excised under the form of **internal eliminated sequences** (**IESs**) that do not make it into the macronucleus. In *O. trifallax*, about 90% of the germline DNA is eliminated, and more than 3500 genes are scrambled by inversion and permutation [52]. DNA elimination is generally viewed as a defense against transposons [41,53–55], and gene scrambling and imprecise excision have been discussed in the context of the generation of somatic diversity [41,56]. We hypothesize that these rearrangements may also have evolved to reinforce the ciliate Weismann barrier. The selective advantage of a single IES would be small for any of the proposed evolutionary drivers, so conceivably, the system emerged as a propensity of the proto-macronucleus to purge an abundant class of transposons, and was fine-tuned afterwards.

Even if the transfer of genetic material from the ciliate macronucleus back to the micronucleus seems impossible [57], the soma can transfer information to the next sexual generation. In animals, the Weismann barrier can be permeable for nongenetic information, and many traits show some degree of nongenetic heritability [58,59]. Examples include epigenetic markings on gamete genomes and the transfer of maternal hormones or antibodies through the placenta or egg yolk (Figure 2). Likewise, the ciliate Weismann barrier is permeable for epigenetic information. During the remodeling of nuclei after sexual reproduction, the chromosome copy numbers in the new macronucleus in *Stylonychia lemnae* [60] and *O. trifallax* [61] are epigenetically regulated by RNA-guides that originate from the old macronucleus. In *T. thermophila*, a change in the structure of the macronucleus can become heritable in a non-Mendelian way: germline-limited sequences that were artificially introduced in the macronucleus were observed to persist in the newly generated macronucleus [62]. The importance of such **transgenerational phenotypic plasticity** remains to be determined, but it might provide a 'peaceful' solution to the potential micronucleus/macronucleus conflict, that is, an evolutionary compromise between the investment in phenotypic plasticity by somatic selection and in germline adaptation.

Concluding remarks

Ciliates are unlike any other unicellular eukaryote. We argue that they present a form of phenotypic plasticity, so far known only in multicellular organisms, based on mechanisms otherwise seen in genetic adaptation. We propose that the framework of phenotypic plasticity by somatic selection (Figures 1 and 2) within a diffuse multicellular organism (Box 1) helps to explain enigmatic ciliate properties such as amitotic nuclear division, the presence of a juvenile and senescent stage, or the intracellular Weismann barrier. Within a single ciliate cell, a mutation can lead to phenotypic plasticity or to genetic adaptation depending on which nucleus it happens in, possibly opening a grey zone between two concepts that are usually separated: plasticity and adaptation. This resonates with recent suggestions that **organismality** may be context-dependent [63], and with ongoing research about the implementation of the Weismann barrier and the evolutionary relevance of somatic genetic variation [64–68]. For such subjects, ciliates could provide a choice

Outstanding questions

Are ciliates the only unicellular organisms capable of somatic selection?

What are the extent and evolutionary importance of the transgenerational transfer of traits acquired by the macronucleus through somatic selection? Transfer of information between sexual generations has been observed, but our knowledge of the communication between nuclei is fragmentary and is limited to only a few species. A specialized form of **genetic assimilation** in which genetic novelties can do a test run in the macronucleus before being acquired by the micronucleus would be a spectacular example of Lamarckian-like adaptation.

Does somatic selection shed light on the evolutionary origin of nuclear dimorphism?

What about the third genome? The mitochondrial genome is under constant selection but without a reset at each sexual generation. This should result in interesting dynamics between short-term coadaptation with the macronucleus, and continuity with the micronucleus.

What are the evolutionary consequences for an organism to harbor a copy of the germline in every cell? Formalizations about the population genetic consequences of having a 'distributed germline' are lacking. It will be important to reconsider concepts such as inclusive fitness, cooperation, and conflict in the context of the organismality of the ciliate clone.

How is the level of macronuclear diversity modulated? In multicellular examples of somatic selection, somatic diversity is under physiological control in order to balance out the costs and benefits of diversity (e.g., only a very specific region of the B lymphocyte genome is rearranged).

experimental model system for hypothesis testing. We hope that the viewpoint that we have put forward here will open stimulating debates on the evolutionary origin of multinucleated cells, the significance of cell–cell interactions, and organismality (see Outstanding questions).

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Declaration of interests

There are no interests to declare.

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