Evolutionary Biology (2018) 45:237–247
https://doi.org/10.1007/s11692-018-9448-9

SYNTHESIS PAPER

Are Planaria Individuals? What Regenerative Biology is Telling Us About the Nature of Multicellularity

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Received: 26 November 2017 / Accepted: 6 March 2018 / Published online: 12 March 2018

Abstract
Freshwater planaria (Platyhelminthes, Turbellaria, Tricladida) pose a challenge to current concepts of biological individuality. We review molecular and developmental evidence suggesting that mature intact planaria are not biological individuals but their totipotent stem cells (neoblasts) are individuals. Neoblasts within a single planarian body are, in particular, genetically heterogeneous, migratory, effectively immortal, and effectively autonomous. They cooperate to maintain the planarian body as an obligate environment but compete to make this environment maximally conducive to the survival of their own neoblast lineages. These results suggest that planaria have not fully completed the transition to multicellularity, but instead represent an intermediate form in which a small number of genetically-heterogeneous, reproductively-competent cells effectively "farm" their reproductively-incompetent offspring.

Keywords Bioelectricity · Cooperation · *Dugesia japonica* · *Dugesia ryukyuensis* · Germ cells · *Girardia tigrina* · Regeneration · *Schmidtea mediterranea* · Stem cells

Introduction

The major evolutionary transitions, including those from prokaryotes to eukaryotes and from free-living cells to multicellularity, all increase the scale over which cooperative interactions dominate competitive interactions (Maynard Smith and Szathmáry 1995, 2015; West et al. 2015). These evolutionary transitions have transformed the biosphere, subjugating the activity of unicellular organisms in favor of the goals of composite entities: multicellular bodies, e.g. of metazoan animals. Free-living cells were incentivized, during these transitions, to cooperate and expand the boundary of the "self," evolving mechanisms to orchestrate their activities toward creation and repair of complex anatomies. The results were new individual entities, with their own reproductive fitness and evolutionary interests, characterized by both larger scales and higher levels of organizational complexity than those of their components. The forces by which these remarkable phase transitions occurred are being probed via approaches from game theory, evolutionary theory, and cell biology. Unraveling the answer is central to developmental biology, the study of primitive cognition, and regenerative bioengineering (Keijzer et al. 2013; Lyon 2006; Pezzulo and Levin 2015).

This view of larger-scale individuality as an outcome of evolutionary transitions toward increased cooperation and decreased competition has led to the replacement of traditional, informal characterizations of "biological individuality" by a new and relatively precise definition of a *biological individual* or *organism* as a living system maintaining both a higher level of internal cooperation and a lower level of internal conflict than either its components or any larger systems of which it is a component (Diaz-Muñoz et al. 2016; Folse and Roughgarden 2010; Queller and Strassmann 2009; Strassmann and Queller 2010; West and Kiers 2009; West et al. 2015). Free-living cells exhibit a higher level of integration and hence cooperation than their components (Fields and Levin 2018) and hence satisfy this criterion; the question of interest in the case of multicellular systems is whether they achieve higher levels of internal cooperation and lower levels of conflict than

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either their component cells or any larger systems—e.g. social groups—of which they are parts.

Hamilton's (1964) rule predicts that cooperation will be maximized when relatedness $r = 1.0$, i.e. when the cooperating entities are members of a clone (cf. Pineda-Krch and Lehtilä 2004; Fisher et al. 2013). Zygotic bottlenecks assure clonality and hence provide a basis for cooperation within multicellular eukaryotes. While Strassmann and Queller (2010) acknowledge that "(t)here are likely to be multicellular organisms that do not go through a single-cell bottleneck" (p. 608) and consider aggregating *Dictyostelium discoideum* as an example (see also Queller and Strassmann 2009; West and Kiers 2009 who also consider this example), discussions of "canonical" multicellular individuals typically assume a zygotic bottleneck. Here we suggest that freshwater planaria (Platyhelminthes, Turbellaria, Tricladida), particularly largely asexual species such as *Dugesia japonica, Dugesia ryukyuensis, Schmidtea mediterranea* and *Girardia tigrina* provide instructive examples of anatomically complex multicellular organisms that reproduce without a zygotic bottleneck. As we will show, these animals raise deep questions about the roles of cooperation and competition in individuality, and about the relationships between stem cells and germ cells both currently and historically.

Following a brief review of the natural history of asexual planaria as it is reproduced in the laboratory, we discuss in turn evidence that planarian totipotent stem cells, termed "neoblasts" (for reviews, see Rossi et al. 2008; Rink 2013; Zhu and Pearson 2016), are genetically heterologous, migratory, effectively immortal, and effectively autonomous. When embedded in their obligate environment—a planarian body or fragment thereof, even one completely lacking other neoblasts—each neoblast is capable of fully regenerating a complete planarian body, via which it reproduces its neoblast progeny. Within their self-constructed and self-maintained environments, therefore, neoblasts behave as biological individuals on the Queller–Strassmann definition. We show using a simulation that high competition between migratory neoblasts can lead to chaotic instability, and suggest that a combination of molecular and bioelectric mechanisms suppress runaway competition. We then consider the generation of neoblasts during embryogenesis in sexual planaria (sexual strains of *Dugesia ryukyuensis* or *Schmidtea mediterranea*) and the differentiation, in turn, of germ cells from progeny of these neoblasts. The lifestyle and regenerative properties of planaria shed light on the plastic line between body and environment. We conclude by hypothesizing that competition between germ and stem cells may have played an important role in metazoan evolution, and may remain a ubiquitous feature of metazoan development with implications for both regenerative medicine and cancer.

Asexual Planaria Reproduce by Proliferation and Differentiation of Neoblasts

Asexual planaria have been a major model system for developmental and regenerative biology for over a century (for reviews, see Durant et al. 2016; Elliott and Sánchez Alvarado 2012; Lobo et al. 2012; Newmark and Sánchez Alvarado 2002). Planaria have a complex anatomy (see Fig. 1) comprising up to 40 distinct cell types (Sánchez Alvarado and Kang 2005). They have well-developed brains with photosensitive eye spots and paired ventral nerve cords (VNCs) that provide dense innervation to the rest of the body (Pagán 2014; Sarnat and Netsky 2002). The nervous system employs both chemical (dopaminergic, serotonergic, octopaminergic and GABAnergic) and electrical [gap junction (GJ)] synapses to support motility, feeding and other behaviors (Rangiah and Palakodeti 2013; for review of earlier work, see; Umesono and Agata 2009). Feeding and defecation employ a motile pharynx and three-lobed blind gut; a distributed system of protonephridia support osmoregulation. Sexual strains are cross-fertilizing hermaphrodites with differentiated ovaries and testes. One of the most remarkable properties of planaria is that any piece is able to regenerate precisely what is missing, and stops when a standard planarian anatomy is achieved (Aboobaker 2011; Durant et al. 2016; Gentile et al. 2011; Owlarn and Bartscherer 2016),

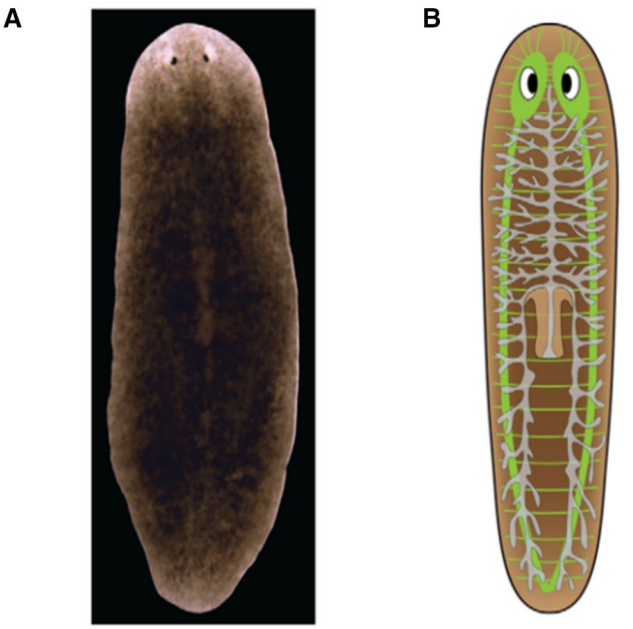

Fig. 1 **a** dorsal view of asexual *D. japonica* showing eyespots; anterior is up. **b** major anatomical structures in asexual Planaria: brain and nervous system in green; excretory system in grey; pharynx in light brown. Adapted from Lobo et al. 2012, Fig. 1. (Color figure online)

making them a popular model system for regenerative medicine research.

Asexual reproduction, the most common mode for many flatworm species, is by fission transverse to the anterior–posterior (A–P) axis followed by regeneration of missing structures. Regeneration requires the presence of stem cells known as neoblasts, which account for approximately 20% of planarian cells. Only neoblasts undergo cell division; all other cells are post-mitotic and turn over in approximately 1 week, in synchrony with their replacement by new differentiated cells (Pellettieri and Sánchez Alvarado 2007). While recent evidence indicates that some neoblasts are committed to a differentiation pathway (Scimone et al. 2014; van Wolfswinkle et al. 2014; Zhu and Pearson 2016), we focus here on totipotent neoblasts, those from which any committed neoblast can be produced. Experiments in which heterologous neoblasts are transplanted into animals that have been sufficiently irradiated to kill all native neoblasts show that a single totipotent neoblast can regenerate a complete animal (Wagner et al. 2011; Zhu and Pearson 2016). Molecular analysis has focused primarily on head/brain and tail regeneration; normal head regeneration is regulated by homologues of mammalian fibroblast (FGF) and epidermal (EGF) growth factors (Agata and Umesono 2008; Fraguas et al. 2014), while tail regeneration is dependent on the *Wnt* pathway (Stückerman et al. 2017; see also; Rink 2013; Owlarn and Bartscherer 2016 for reviews of additional pathways). Importantly, key aspects of regenerative response, including size control and anterior–posterior organ identity, are also dependent on signaling by bioelectric pathways (Levin et al. 2017; Levin and Martyniuk 2017). A primary component of this bioelectric signaling is cell–cell communication via GJ; disrupting GJ leads to two-headed regenerates (Oviedo et al. 2010). Recent work has shown that tail regeneration is dependent on hyperpolarization of the posterior wound; "cryptic" regenerates of GJ-blocked animals have normal anatomy but depolarized tails, and produce two-headed regenerates after amputation at a constant ratio for multiple generations (Durant et al. 2017).

The multiple manipulations that produce regenerates with two well-formed heads housing functional brains and the relative paucity of manipulations that produce headless animals with two well-formed tails suggests that brain and head production in response to a wound cutting across the A–P axis is a default for neoblasts (Lobo and Levin 2015). This in turn suggests that asexual reproduction in planaria is a finally tuned system that not only prevents A–P symmetric, two-headed regeneration but also enforces asymmetry along the dorso-ventral and medio-lateral axes to regenerate a complete anatomy with appropriately sized and placed organs and external morphology.

Planaria raise several fascinating conundrums that challenge our understanding of the relationships between the genome and body anatomy. In most advanced organisms, Weissman's barrier ensures that somatic mutations do not propagate to offspring. In planaria, however, any mutation that does not kill a neoblast is propagated into subsequent generations. As will be seen below, the planarian genome bears clear evidence of this chaotic process. And yet, despite hundreds of millions of years of accumulating somatic mutations, planarian regenerative anatomy exhibits almost 100% fidelity—each regenerating planarian is a perfect, normal copy of the standard planarian target morphology. How can the anatomy stay constant and robust while the genome diverges? Interestingly, in contrast to other model species (mouse, *C. elegans, Drosophila*, zebrafish, etc.) in which patterning mutants are plentiful, there is only one known strain of planaria that permanently propagates an unusual anatomy: the two-headed forms induced by perturbation of communication among planarian stem cells and soma (Oviedo and Levin 2007; Oviedo et al. 2010; Nogi and Levin 2005). Planaria are also effectively immortal—no evidence of aging at the level of the individual animal has been documented in species like *D. japonica*. Given these unusual properties, we explored the implications of planarian biology for understanding the forces that define biological individuality.

Neoblasts Satisfy Criteria for Biological Individuality

Neoblasts are Genetically Heterologous

Any population that reproduces asexually can be expected to exhibit genetic heterogeneity due to somatic mutations. As only neoblasts are mitotic in planaria, any genetic heterogeneity due to somatic mutation must be transmitted along neoblast lineages. Selection pressure would, therefore, be expected to act against somatic mutation in these lineages to maintain a more genetically homogeneous population.

Planaria have long been known to be mixoploid (Newmark and Sánchez Alvarado 2002). Hoshino et al. (1991), for example, found di-, tri- and tetraploid cells in *D. japonica* using flow cytometry; many other groups have reported similar observations. More recently, Ermakov et al. (2012) were able to isolate di-, tri-, tetra- and hexaploid neoblasts from *G. tigrina* and di- and tetraploid neoblasts from *S. mediterranea*, again with flow cytometry. Knakievicz et al. (2007) demonstrated both mixoploidy and considerable heterogeneity of ploidy across isolates in wild populations from 16 sites in southern Brazil.

The genomes within these karyotypically heterologous neoblasts appear to be highly heterologous at the DNA sequence level. Nishimura et al. (2015) performed both genomic DNA and cDNA sequence analysis on libraries constructed from a 20-year-old clonal *D. japonica* colony produced by exclusively asexual reproduction from a single

founder individual. They observed non-synonymous base substitutions in the coding regions of 74% of predicted genes. It is worth emphasizing that the planaria employed in this study were morphologically normal and otherwise apparently wild type. While this result clearly requires extension to other planarian species to be considered general, it is consistent with the maintenance of a well-defined wild type morphology and behavior in lineages of animals that have undergone asexual reproduction for many thousands of generations.

Neoblasts are Migratory

The extent to which neoblasts are migratory has been controversial, with experimental results obtained by Saló and Baguñà (1985) contradicting earlier claims of wound-directed motility. More recently, however, Guedelhoefer and Sánchez Alvarado (2012) have shown that while neoblasts do not migrate in response to lethal irradiation of part of the animal, they do migrate in response to wounding. Abnave et al. (2017) show that neoblast migration requires new transcription and is responsive to signals within intact animals as well as to wounding.

Neoblast migration can be expected to contribute to neoblast genetic heterogeneity, as shown in Fig. 2. Reproduction by fission generates wounds to which neoblasts migrate. The neoblasts at a wound site contribute progeny to the regenerated structures, which are then available to migrate to subsequent wound sites and contribute progeny to subsequent regenerated structures. Any particular worm can be expected to carry neoblasts from many distinct neoblast lineages, in proportions that may differ from those found in any other worm.

Neoblasts are Effectively Immortal

While both morphological stability and degrowth in the absence of adequate food supplies indicate that neoblasts are subject to regulated cell death (Pellettieri and Sánchez Alvarado 2007), neoblast lineages appear to be effectively immortal. The results of Nishimura et al. (2015) show that single neoblast lineages can survive for at least 20 years; the survival of multiple clonal colonies in laboratories around the world confirms this, and the long-term survival of asexual populations in the wild, apparently without periodic sexualization, suggests that neoblast lineage survival is indefinite.

Planarian neoblasts can be successfully transplanted between genetically-distinct sexual and asexual strains (e.g. Wagner et al. 2011; Guedelhoefer and Sánchez Alvarado

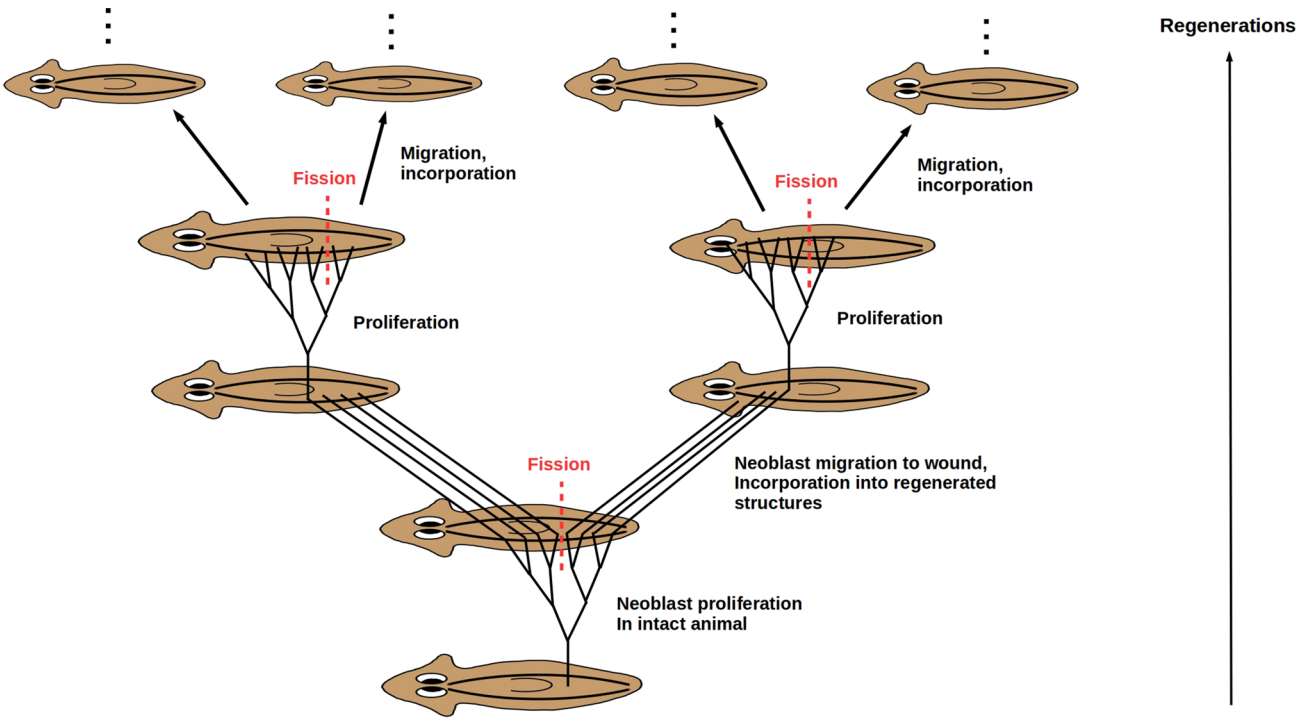

Fig. 2 Distribution of neoblasts from a single lineage into a population produced by regeneration. The lineage proliferates in intact animals. At each fission event, neoblasts migrate to the wound surfaces and their neoblast as well as non-neoblast progeny are incorporated into the regenerated structures. Neoblast migration to wound sites contributes to neoblast genetic heterogeneity, as progeny of many neoblast lineages migrate to wounds and hence contribute to the neoblast populations of the regenerated structures. (Color figure online)

2012). Neoblasts were effectively transplanted in early twentieth century xenografting experiments by T. H. Morgan and others, but these have not been repeated with currently-available neoblast labeling technologies and their interpretation remains unclear (reviewed by Zattara 2015). Neoblasts do not survive well in vitro (Schürmann and Peter 2001), suggesting that a functioning planarian body may be their obligate environment. A functioning planarian body appears to have been, at any rate, their obligate environment during their evolutionary history to date.

Neoblasts are Effectively Autonomous

As noted above, single neoblast transplantation following lethal radiation shows that a single totipotent neoblast can regenerate a complete animal (Wagner et al. 2011; Zhu and Pearson 2016). When embedded in the right environment, therefore, neoblasts can act autonomously, dividing to produce a clone of daughter neoblasts that then divide to produce clones of differentiated cells.

Neoblasts respond to a wide variety of signaling molecules, including Wnt, Hedgehog, TGF-β, Netrin, FGF and EGF family signals (Elliott and Sánchez Alvarado 2012; Fraguas et al. 2011; Rink 2013), as well as to endogenous bioelectric signals that dictate which structures the neoblasts should help build (Beane et al. 2011, 2013; Durant et al. 2017; Emmons-Bell et al. 2015). Although the sources and specific roles of these signals have yet to be fully characterized, the roles of these signals in initiating and/or modulating wound response, defining polarity along body axes, and regulating differentiation strongly suggest that they are generated by differentiated or differentiating cells, not by other neoblasts. The extent to which neoblasts communicate directly amongst themselves is unknown.

Are Neoblasts Individuals?

As seen above, planarian totipotent neoblasts exhibit common characteristics of individuality. They clearly satisfy, moreover, the Queller–Strassmann definition of biological individuals as systems that maintain a high level of internal cooperation while minimizing internal conflict. Is it reasonable, therefore, to consider them individuals? We suggest that it is reasonable, and indeed that it is more reasonable, on the basis of Hamilton's rule as well as their behavior, to consider single neoblasts as individuals than to consider either populations of neoblasts or the bodies that contain them as individuals. The latter groups not only have relatedness $r < 1$, they exhibit less internal cooperation and more internal conflict than do single neoblasts. Hence if the goal is identify a *single* level of organization at which cooperation is maximized and competition minimized (Queller and Strassmann 2009; Strassmann and Queller 2010) in asexual planaria, it is the level of the neoblast.

If planarian neoblasts are individuals, they are individuals of quite an interesting type. They are, in particular, individuals that inhabit an obligate, high-complexity environment that they construct entirely out of their own reproductively-incompetent progeny. They resemble, in this sense, reproductive queens inhabiting colonies of sterile workers, all of which are their descendants. The presence of multiple, genetically heterologous neoblasts within a single planarian body, however, causes any strict analogy along these lines to break down. Any given neoblast and the body within which it lives exhibit mutual complete reproductive dependency, but the heterologous population of neoblasts inhabiting a given body do not exhibit mutual complete reproductive dependency; indeed they are reproductively independent. Neoblasts therefore violate Fisher et al.'s (2013) extension of Boomsma's hypothesis; they cooperate in maintaining their shared environment although they are not equally related to each other or to the non-reproductive offspring that compose that environment. To the extent that genetic variants among neoblasts within a planarian body lead to differences in responsiveness to inter- or intracellular signals, cell-cycle rate or metabolic efficiency—all differences that may be expected given the extreme coding-sequence diversity observed by Nishimura et al. (2015)—neoblasts and neoblast lineages may be expected to compete as well as cooperate in the context of a single planarian body.

From the perspective of a single neoblast, "reproduction" at the scale of the planarian body is expansion of its obligate environment. All extant planarians within an asexual lineage, e.g. all extant asexual *D. japonica* can, therefore, be viewed as the single specialized ecological niche of a highly-heterologous population of reproductively-independent biological individuals, the extant asexual *D. japonica* neoblasts. These individuals share a genetic interest in maintaining and expanding this niche indefinitely. They also have potentially-conflicting genetic interests in making that shared environment as conducive as possible to their own, and their lineages', reproductive success. As any given neoblast lineage occupies many dispersed parts of this environment—i.e. many planarian bodies—loss or reproductive failure of any particular planarian body has little impact on the reproductive prospects of the neoblast lineages occupying it. While planarian bodies may look and behave like independent reproductive units, they are in an important sense neither independent nor reproductive units. They do not, moreover, minimize internal competition, though as will be discussed below they must moderate it somewhat; therefore they do not satisfy the Queller–Strassmann definition of individuality as characterizing the level of organization at which cooperation is maximized and competition minimized. Hence we suggest

that the question posed by the title be answered in the negative in the case of asexual planaria.

Competition Between Functionally Heterologous Neoblasts can Lead to Instability

While the heterologous neoblasts occupying a planarian body can be expected to compete for the reasons outlined above, this competition must be moderated in a way that prevents uncontrolled growth or resource monopolization by particular lineages (Aktipis et al. 2015). Planaria share major tumor-suppressor gene families, including p53 and PTEN, with mammals (Oviedo and Beane 2009; Pearson and Sánchez Alvarado 2008), suggesting that tumor-suppression pathways are involved in growth control. How control is implemented at the level of individual neoblast lineages is, however, not yet understood. The occurrence of spontaneous tumors in multiple planarian species (reviewed by Aktipis et al. 2015) indicates that it is not always successful.

Two potential mechanisms for moderating competition between neoblast lineages are to limit inter-neoblast competition per se and to limit the extent to which local cellular environments are more conducive to growth by neoblasts of their parent versus other lineages. We have developed a simple agent-based model to examine these mechanisms both individually and in combination; the model can be manipulated and its source code examined at https://chrisfieldsresearch.com/neoblast-competition-v2.htm. We consider a population of neoblasts of different lineages randomly embedded within a population of non-neoblast cells (Fig. 3a), and model both local cellular turnover and neoblast migration. If migration is not allowed, the model maintains a random lineage distribution; a non-random initial state would, with no migration, simply maintain its initial state. Migration in the model provides a representation of differences in fitness between neoblast lineages. More fit lineages expand to occupy additional territory in the model space (Fig. 3b).

The model provides two more sensitive ways of manipulating relative fitness: imposing regional survival biases in favor of particular neoblast populations and against others and increasing the competitive advantage (effectively, reproductive rate) of "fitter" neoblasts within those regions. The imposition of even absolute (100%) regional biases for migration is compatible with stable outcomes (Fig. 3c) provided the competitive advantage of fitter neoblasts is kept only 20% higher than that of other neoblasts. Increasing the

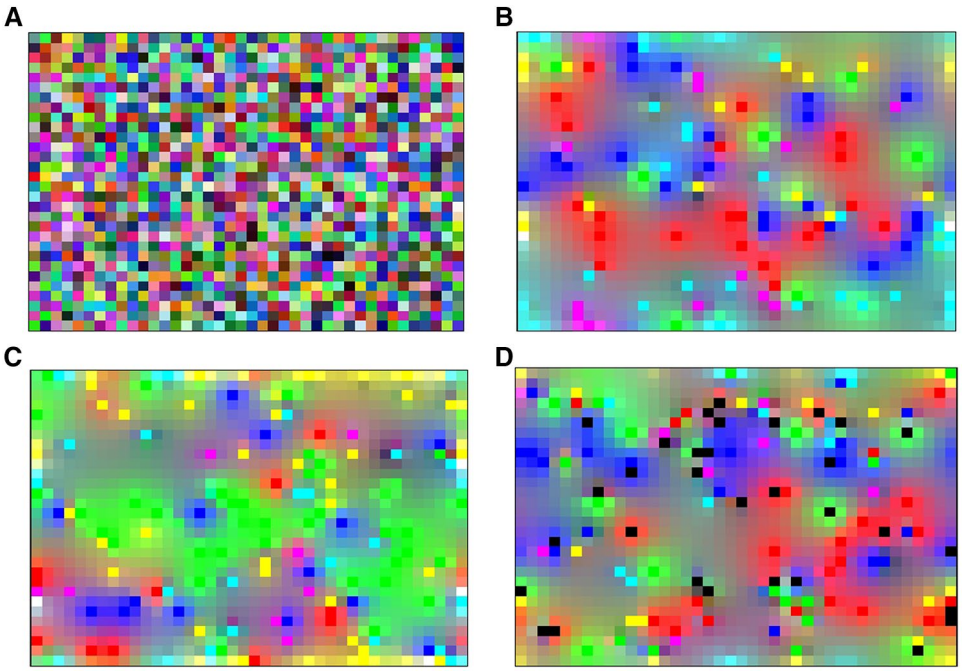

Fig. 3 **a** Typical initial model state, representing a random embedding of neoblasts of different lineages in a random non-neoblast background. Squares are model "cells" representing small volumes of the planarian body. Red, green, blue, magenta, cyan and yellow colors represent single pure neoblast lineages; intermediate colors are mixtures with gray representing an equal mixture of the six lineages. **b** Final state producing by allowing migrations with 10% probability for 120 time steps from the initial state on the left. Progressively increasing the migration probability leads to a progressively "grayer" more uniform outcomes. **c** Typical final state following 10% migration with absolute regional biases. **d** Unstable outcome of winner-take-all competition. Black squares are "dead" model cells in which all lineages have been forced to zero population. See https://chrisfieldsresearch.com/neoblast-competition-v2.htm for further details and to manipulate the model. (Color figure online)

competitive advantage of fitter neoblasts to 60% (with no lineage-specific survival bias and other model parameters at default settings), however, produces unstable winner-take-all competition in which some regions rapidly alternate between dominant lineages or between some dominant lineage and cell death (Fig. 3d). Imposing regional survival biases (effectively, differences in local environmental compatibility) in favor of particular populations and against others similarly produces unstable behavior. These parameters interact over a substantial range, as shown in Fig. 4.

We conclude from experiments with this model that competition between neoblast lineages must be actively suppressed above some maximal value consistent with anatomical, morphological and behavioral stability. In particular, competition must remain sufficiently suppressed that it does not interfere with either the the behaviors required for asexual fission or the regenerative processes required to replace missing organs and systems. The facility with which tumors can be induced by RNAi inhibition of homologues of mammalian tumor-suppressor genes (Oviedo and Beane 2009; Pearson and Sánchez Alvarado 2008) is consistent with active suppression of runaway reproductive competition. The apparent reversion of tumors to normal tissue in the course of regeneration (Seilern-Aspang and Kratochwil 1965) suggests that this suppression of competition is particularly strong during the regeneration process.

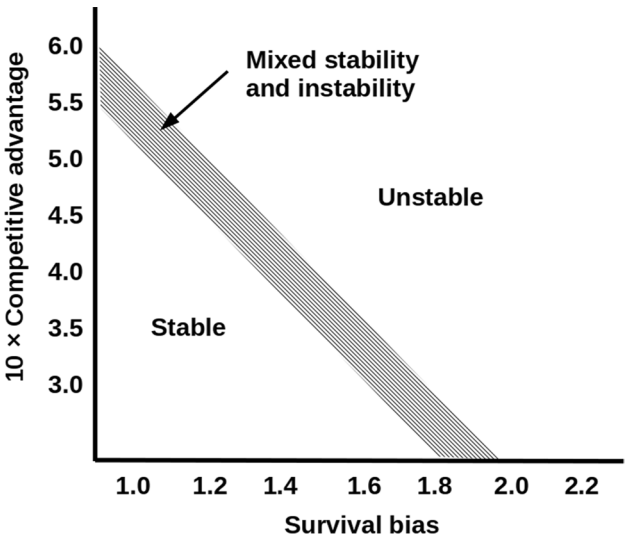

Fig. 4 Regions of stable, unstable and mixed model outcomes based on 200 120-step model runs with varying competitive advantage and survival bias values (all other parameters at default values). Either type of bias in favor of some lineages over others leads to winner-take-all competition and instability, e.g. rapidly varying dominant lineages or areas of cell death

Neoblasts and Germ Cells have Competing Interests

Sexual planaria are cross-fertilizing hermaphrodites with differentiated ovaries, testes, yolk glands, oviducts and copulatory apparatus (Hoshi et al. 2003). As in asexual planaria, all adult structures are composed of differentiated progeny of neoblasts. Embryogenesis proceeds through two phases, the differentiation of temporary embryonic structures and their later complete replacement by adult structures (reviewed by Martín-Durán et al. 2012). All embryonic structures are formed by progeny of *piwi-1* expressing blastomeres that by the initiation of adult-structure differentiation are identifiable morphologically and molecularly as neoblasts (Davies et al. 2017). The neoblast population of a sexual lineage can, therefore, be viewed as alternating with the germline and zygote in a continuous cycle that produces non-germ, non-neoblast somatic cells as products (Solana 2013; Petralia et al. 2014); Fig. 5 depicts this cycle in simplified form.

Asexual planaria can be sexualized by feeding them sexual planaria (Hoshi et al. 2003; Nodono et al. 2012) or by transplanting neoblasts from sexual planaria into them (Nodono et al. 2012; Guedelhoefer and Sánchez Alvarado 2012). Sexualized neoblasts capable of differentiation to produce germ cells are, therefore, in some sense dominant over asexual neoblasts not competent to produce germ cells. They are, in particular, able to suppress the immortality of asexual neoblasts by forcing the organism-scale reproductive process through a

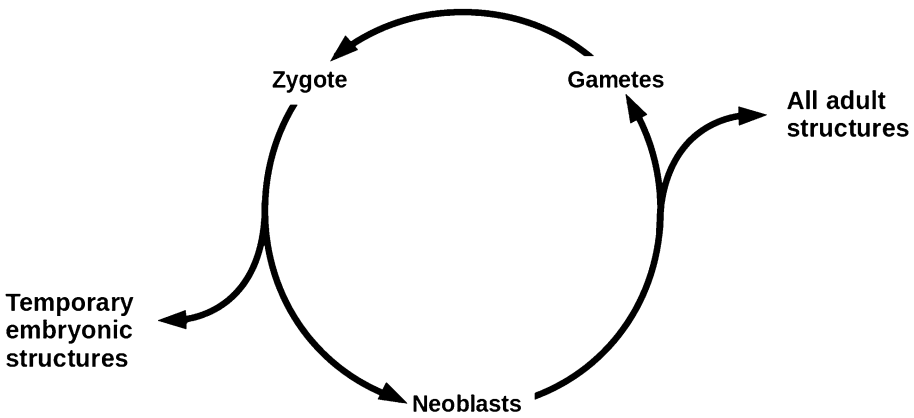

Fig. 5 Neoblast-to-germline cycle in sexual planaria. The Weissman barrier separates non-germ, non-neoblast somatic cells from the neoblast-to-germline cycle (Solana 2013; Petralia et al. 2014)

zygotic bottleneck that only they can initiate and only their lineage can survive. This suppression is not always complete, as at least some sexual or sexualized planaria continue to reproduce asexually under favorable conditions.

Both the broad phylogenetic distribution of regenerative capabilities in multicellular animals and the use of similar molecular pathways for wound healing and regeneration across animal phylogeny suggest that regeneration is ancestral (Rink 2013; Fumagalli et al. 2017; Kenny et al. 2017), although the question of multiple origins of regenerative capability in animals remains open (Bely 2010; Tiozzo and Copley 2016). The evolutionary relationship between sexuality and regenerative ability similarly remains open. While detailed studies have yet to be undertaken in flatworms, phylogenetic analysis of regeneration and sexuality in annelids suggests that both regenerative ability and sexuality are ancestral but asexual reproduction is derived (Zattara and Bely 2016). Loss of regenerative ability in the planarian *Procotyla fluviatilis* has been linked to dysregulation of Wnt signalling (Sikes and Newmark 2013), consistent with the ubiquitous involvement of the Wnt pathway in animal regeneration across phylogeny.

Competition between sexual, germline-competent and asexual, germline-incompetent neoblasts for control of reproduction is analogous to the competition between germline and somatic cells that characterizes obligately-sexual organisms. Such competition, when combined with the autonomy of asexual neoblasts within the planarian body, challenges mutualist aggregation-based models of multicellularity, whether "fraternal" or "egalitarian" (Strassmann and Queller 2010), in favor of a model in which the key step is the suppression of reproductive competence in progeny, after which they are effectively farmed for resources. Germ cells exemplify this "imperial" style of multicellularity even more than non-germ stem calls, including totipotent ones such as asexual planarian neoblasts, as the former effectively suppress the immortality of the latter. An imperial model of multicellularity similarly challenges organism-scale theories of sex (reviewed by Otto and Lenormand 2002) by suggesting that sex may be viewed as an outcome of a successful revolt of one stem-cell population against others. Such a view suggests, in turn, that gonads may be actively involved post-reproduction in triggering organismal senescence. The regulation of resource allocation in short-lived cephalopod species pre- and post-reproduction (e.g. Moltschaniwskyj and Carter 2013) may provide a useful model system for addressing this hypothesis.

Conclusion

Asexual planaria appear, on the basis of morphology, behavior and lifecycle, to be autonomous biological individuals. We have reviewed molecular and developmental evidence that this appearance is deceiving: asexual planarian bodies are genetically heterozygous assemblages of reproductively-incompetent cells that are inhabited and maintained, as an obligate environment, by populations of genetically heterologous, migratory, effectively immortal, and effectively autonomous stem cells, the asexual planarian neoblasts. These neoblasts cooperate in maintaining the planarian body, but compete for its resources and its conduciveness to their own genetic lineage. How inter-neoblast cooperation sufficient to maintain morphological and behavioral integrity and indeed constancy over thousands of asexual generations is enforced remains unknown. Asexual planarian bodies can be taken over by sexual neoblast lineages that force organism-scale reproduction through a zygotic bottleneck that only their lineages can survive.

We suggest that these features of planaria make them a useful model system for evolutionary as well as developmental biology. These organisms appear, in particular, not yet to have fully completed the transition to multicellular individuality. They appear, instead, to be intermediate forms in which internal cooperation is sufficient to generate a well-defined morphology and a complex, coordinated anatomy but internal competition is still physiologically and reproductively significant. As many "lower" invertebrates have totipotent stem cells functionally analogous to planarian neoblasts (Rink 2013), incomplete transitions to multicellularity may be commonplace in the metazoa. We might speculate that such animals can display a combination of striking regenerative abilities and relatively low rates of spontaneous tumor formation in part because their stem cells are "imperial" in the sense of completely suppressing the reproduction of their non-stem-cell progeny.

Planaria raise, but we cannot yet answer, interesting questions about the origins of morphological asymmetry and sex. Mechanisms regulating body axis definition and polarity, including bioelectricity, are both ancient and highly conserved in eukaryotes (reviewed by Fields and Levin 2018); however, the transition from symmetric to asymmetric forms remains poorly understood. The extent to which sex may represent an "imperial" takeover of organism-scale reproduction by a select population of stem cells remains to be investigated.

Acknowledgements We thank A. Aboobaker for useful discussions. This research was supported by the Allen Discovery Center program through The Paul G. Allen Frontiers Group (12171). In addition, M. L. gratefully acknowledges support of the G. Harold and Leila Y. Mathers Charitable Foundation (TFU141) and the Templeton World Charity Foundation (TWCF0089/AB55 and TWCF0140).

Compliance with Ethical Standards

Conflict of interest The authors declare that they are aware of no potential conflicts, financial or otherwise, pertaining to this work.

References

Abnave, P., Aboukhatwa, E., Kosaka, N., Thompson, J., Hill, M. A., & Aboobaker, A. A. (2017). Epithelial-mesenchymal transition transcription factors control pluripotent adult stem cell migration in vivo in planarians. *Development, 144*, 3440–3453. https://doi.org/10.1242/dev.154971.

Aboobaker, A. A. (2011). Planarian stem cells: A simple paradigm for regeneration. *Trends in Cell Biology, 21*, 304–311. https://doi.org/10.1016/j.tcb.2011.01.005.

Agata, K., & Umesono, Y. (2008). Brain regeneration from pluripotent stem cells in planarian. *Philosophical Transactions of the Royal Society B, 363*, 2071–2078. https://doi.org/10.1098/rstb.2008.2260.

Aktipis, C. A., Boddy, A. M., Jansen, G., Hibner, U., Hochberg, M. E., Maley, C. C., & Wilkinson, G. S. (2015). Cancer across the tree of life: Cooperation and cheating in multicellularity. *Philosophical Transactions of the Royal Society B, 370*, 20140219. https://doi.org/10.1098/rstb.2014.0219.

Beane, W. S., Morokuma, J., Adams, D. S., & Levin, M. (2011). A chemical genetics approach reveals H,K-ATPase-mediated membrane voltage is required for planarian head regeneration. *Chemistry & Biology, 18*, 77–89. https://doi.org/10.1016/j.chembiol.2010.11.012.

Beane, W. S., Morokuma, J., Lemire, J. M., & Levin, M. (2013). Bioelectric signaling regulates head and organ size during planarian regeneration. *Development, 140*, 313–322. https://doi.org/10.1242/dev.086900.

Bely, A. E. (2010). Evolutionary loss of animal regeneration: Pattern and process. *Integrative and Comparative Biology, 50*(4), 515–527. https://doi.org/10.1093/icb/icq118

Davies, E. L., Lei, K., Seidel, C. W., Kroesen, A. E., McKinney, S. A., Guo, L., Robb, S. M. C., Ross, E. J., Gotting, K., & Sánchez Alvarado, A. (2017). Embryonic origin of adult stem cells required for tissue homeostasis and regeneration. *eLife, 6*, e21052. https://doi.org/10.7554/eLife.21052.

Diaz-Muñoz, S. L., Boddy, A. M., Dantas, G., Waters, C. M., & Bronstein, J. L. (2016). Contextual organismality: Beyond pattern to process in the emergence of organisms. *Evolution, 70*(12), 2669–2677. https://doi.org/10.1111/evo.13078.

Durant, F., Lobo, D., Hammelman, J., & Levin, M. (2016). Physiological controls of large-scale patterning in planarian regeneration: A molecular and computational perspective on growth and form. *Regeneration, 3*(2), 78–102. https://doi.org/10.1002/reg2.54.

Durant, F., Morokuma, J., Fields, C., Williams, K., Adams, D. A., & Levin, M. (2017). Long-term, stochastic editing of regenerative anatomy via targeting endogenous bioelectric gradients. *Biophysical Journal, 112*, 2231–2243. https://doi.org/10.1016/j.bpj.2017.04.011.

Elliott, S. A., & Sánchez Alvarado, A. (2012). The history and enduring contributions of planarians to the study of animal regeneration. *Wiley Interdisciplinary Reviews: Developmental Biology, 2*(3), 301–326. https://doi.org/10.1002/wdev.82.

Emmons-Bell, M., Durant, F., Hammelman, J., Bessonov, N., Volpert, V., Morokuma, J., Pinet, K., Adams, D. S., Pietak, A., Lobo, D., & Levin, M. (2015). Gap junctional blockade stochastically induces different species-specific head anatomies in genetically wild-type *Girardia dorotocephala* flatworms. *International Journal of Molecular Sciences, 16*, 27865–27896. https://doi.org/10.3390/ijms161126065.

Ermakov, A. M., Ermakov, O. N., Kudravtsev, A. A., & Kreshchenko, N. D. (2012). Study of planarian stem cell proliferation by means of flow cytometry. *Molecular Biology Reports, 39*(3), 3073–3080. https://doi.org/10.1007/s11033-011-1070-1.

Fields, C., & Levin, M. (2018). Multiscale memory and bioelectric error correction in the cytoplasm–cytoskeleton-membrane system. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 10*(2), e1410. https://doi.org/10.1002/wsbm.1410.

Fisher, R. M., Cornwallis, C. K., & West, S. A. (2013). Group formation, relatedness, and the evolution of multicellularity. *Current Biology, 23*(12), 1120–1125. https://doi.org/10.1016/j.cub.2013.05.004.

Folse, H. J. III, & Roughgarden, J. (2010). What is an individual organism? A multilevel selection perspective. *The Quarterly Review of Biology, 85*(4), 447–472. https://doi.org/10.1086/656905.

Fraguas, S., Barberán, S., & Cebrià, F. (2011). EGFR signaling regulates cell proliferation, differentiation and morphogenesis during planarian regeneration and homeostasis. *Developmental Biology, 354*, 87–101. https://doi.org/10.1016/j.ydbio.2011.03.023.

Fraguas, S., Barberán, S., Iglesias, M., Rodríguez-Esteban, G., & Cebrià, F. (2014). *egr-4*, a target of EGFR signaling, is required for the formation of the brain primordia and head regeneration in planarians. *Development, 141*, 1835–1847. https://doi.org/10.1242/dev.101345.

Fumagalli, M. R., Zapperi, S., & La Porta, C. A. M. (2017). Regeneration in distantly related species: Common strategies and pathways. *NPJ Systems Biology and Applications*. https://doi.org/10.1038/s41540-017-0042-z.

Gentile, L., Cebria, F., & Bartscherer, K. (2011). The planarian flatworm: An in vivo model for stem cell biology and nervous system regeneration. *Disease Models & Mechanisms, 4*, 12–19. https://doi.org/10.1242/dmm.006692.

Guedelhoefer, O. C., & Sánchez Alvarado, A. (2012). Amputation induces stem cell mobilization to sites of injury during planarian regeneration. *Development, 139*, 3510–3520. https://doi.org/10.1242/dev.082099.

Hamilton, W. D. (1964). The genetical theory of social behavior. *Journal of Theoretical Biology, 7*(1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4.

Hoshi, M., Kobayashi, K., Arioka, S., Hase, S., & Matsumoto, M. (2003). Switch from asexual to sexual reproduction in the planarian *Dugesia ryukyuensis*. *Integrative and Comparative Biology, 43*, 242–246.

Hoshino, K., Ohnisji, K., Yoshida, W., & Shinozawa, T. (1991). Analysis of ploidy in a planarian by flow cytometry. *Hydrobiologia, 227*(1), 175–178. https://doi.org/10.1093/icb/43.2.242.

Keijzer, F., van Duijn, M., & Lyon, P. (2013). What nervous systems do: Early evolution, input-output, and the skin brain thesis. *Adaptive Behavior, 21*, 67–85. https://doi.org/10.1177/1059712312465330.

Kenny, N. J., de Goeij, J. M., de Bakker, D. M., Whalen, C. G., Berezikov, E., & Riesgo, A. (2017). Towards the identification of ancestrally shared regenerative mechanisms across the Metazoa: A Transcriptomic case study in the Demosponge *Halisarca caerulea*. *Marine Genomics*. https://doi.org/10.1016/j.margen.2017.11.001.

Knakievicz, T., Lau, A. H., Prá, D., & Erdtmann, B. (2007). Biogeography and karyotypes of freshwater planarians (Platyhelminthes, Tricladida, Paludicola) in southern Brazil. *Zoological Science, 24*, 123–129. https://doi.org/10.2108/zsj.24.123.

Levin, M., & Martyniuk, C. J. (2017). The bioelectric code: An ancient computational medium for dynamic control of growth and form. *Biosystems* in press. https://doi.org/10.1016/j.biosystems.2017.08.009.

Levin, M., Pezzulo, G., & Finkelstein, J. M. (2017). Endogenous bioelectric signaling networks: Exploiting voltage gradients for control of growth and form. *Annual Review of Biomedical Engineering, 19*, 353–387. https://doi.org/10.1146/annurev-bioeng-071114-040647.

Lobo, D., Beane, W. S., & Levin, M. (2012). Modeling planarian regeneration: A primer for reverse-engineering the worm. *PLoS Computational Biology, 8*(4), ee1002481. https://doi.org/10.1371/journal.pcbi.1002481.

Lobo, D., & Levin, M. (2015). Inferring regulatory networks from experimental morphological phenotypes: A computational method reverse-engineers planarian regeneration. *PLoS Computational Biology, 11*(6), e1004295.

Lyon, P. (2006). The biogenic approach to cognition. *Cognitive Processing, 7*, 11–29. https://doi.org/10.1007/s10339-005-0016-8.

Martín-Durán, J. M., Monjo, F., & Romero, R. (2012). Planarian embryology in the era of comparative developmental biology. *International Journal of Developmental Biology, 56*, 39–48. https://doi.org/10.1387/ijdb.113442jm.

Maynard Smith, J., & Szathmáry, E. (1995). *The major transitions in evolution*. Oxford: W. H. Freeman.

Moltschaniwskyj, N. A., & Carter, C. G. (2013). The adaptive response of protein turnover to the energetic demands of reproduction in a cephalopod. *Physiological and Biochemical Zoology, 86*, 119–126. https://doi.org/10.1086/667799.

Newmark, P. A., & Sánchez Alvarado, A. (2002). Not your father's planarian: A classic model enters the era of functional genomics. *Nature Reviews Genetics, 3*, 210–219. https://doi.org/10.1038/nrg759.

Nishimura, O., Hosoda, K., Kawaguchi, E., Yazawa, S., Hayashi, T., Umesono, Y., & Agata, K. (2015). Unusually large number of mutations in asexually reproducing clonal planarian *Dugesia japonica*. *PLoS ONE, 10*(11), e0143525. https://doi.org/10.1371/journal.pone.0143525.

Nodono, H., Ishino, Y., Hoshi, M., & Matsumoto, M. (2012). Stem cells from innate sexual but not acquired sexual planarians have the capability to form a sexual individual. *Molecular Reproduction and Development, 79*, 757–766. https://doi.org/10.1002/mrd.22109.

Nogi, T., & Levin, M. (2005). Characterization of innexin gene expression and functional roles of gap-junctional communication in planarian regeneration. *Developmental biology, 287*(2), 314–335. https://doi.org/10.1016/j.ydbio.2005.09.002.

Otto, S. P., & Lenormand, T. (2002). Resolving the paradox of sex and recombination. *Nature Reviews Genetics, 3*, 252–261. https://doi.org/10.1038/nrg761.

Oviedo, N. J., & Beane, W. S. (2009). Regeneration: The origin of cancer or a possible cure? *Seminars in Cell & Developmental Biology, 20*, 557–564. https://doi.org/10.1016/j.semcdb.2009.04.005.

Oviedo, N. J., & Levin, M. (2007). *smedinx-11* is a planarian stem cell gap junction gene required for regeneration and homeostasis. *Development, 134*, 3121–3131. https://doi.org/10.1242/dev.006635.

Oviedo, N. J., Morokuma, J., Walentek, P., Kema, I. P., Gu, M. B., Ahn, J.-M., Hwang, J. S., Gojobori, T., & Levin, M. (2010). Long-range neural and gap junction protein-mediated cues control polarity during planarian regeneration. *Developmental Biology, 339*, 188–199. https://doi.org/10.1016/j.ydbio.2009.12.012.

Owlarn, S., & Bartscherer, K. (2016). Go ahead, grow a head! A planarian's guide to anterior regeneration. *Regeneration, 3*(3), 139–155. https://doi.org/10.1002/reg2.56.

Pagán, O. R. (2014). *The first brain: The neuroscience of planarians*. Oxford: Oxford University Press.

Pearson, B. J., & Sánchez Alvarado, A. (2008). Regeneration, stem cells, and the evolution of tumor suppression. *Cold Spring Harbor Symposia on Quantitative Biology, 73*, 565–572. https://doi.org/10.1101/sqb.2008.73.045.

Pellettieri, J., & Sánchez Alvarado, A. (2007). Cell turnover and adult tissue homeostasis: From humans to planarians. *Annual Review of Genetics, 41*, 83–105. https://doi.org/10.1146/annurev.genet.41.110306.130244.

Petralia, R. S., Mattson, M. P., & Yao, P. J. (2014). Aging and longevity in the simplest animals and the quest for immortality. *Ageing Research Reviews, 16*, 66–82. https://doi.org/10.1016/j.arr.2014.05.003.

Pezzulo, G., & Levin, M. (2015). Re-membering the body: Applications of computational neuroscience to the top-down control of regeneration of limbs and other complex organs. *Integrative Biology, 7*, 1487–1517. https://doi.org/10.1039/C5IB00221D.

Pineda-Krch, M., & Lehtilä, K. (2004). Costs and benefits of genetic heterogeneity within organisms. *Journal of Evolutionary Biology, 17*(6), 1167–1177. https://doi.org/10.1111/j.1420-9101.2004.00808.x.

Queller, D. C., & Strassmann, J. E. (2009). Beyond society: The evolution of organismality. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1533), 3143–3155. https://doi.org/10.1098/rstb.2009.0095.

Rangiah, K., & Palakodeti, D. (2013). Comprehensive analysis of neurotransmitters from regenerating planarian extract using an ultrahigh-performance liquid chromatography/mass spectrometry/selected reaction monitoring method. *Rapid Communications in Mass Spectrometry, 27*, 2439–2452. https://doi.org/10.1002/rcm.6706.

Rink, J. C. (2013). Stem cell systems and regeneration in planaria. *Development Genes and Evolution, 223*(1–2), 67–84. https://doi.org/10.1007/s00427-012-0426-4.

Rossi, L., Salvetti, A., Batistoni, R., Deri, P., & Gremigni, V. (2008). Planarians, a tale of stem cells. *Cellular and Molecular Life Sciences, 65*, 16–23. https://doi.org/10.1007/s00018-007-7426-y.

Saló, E., & Baguñà, J. (1985). Cell movement in intact and regenerating planarians. Quantitation using chromosomal, nuclear and cytoplasmic markers. *Journal of Embryology and Experimental Morphology, 89*, 57–70.

Sánchez Alvarado, A., & Kang, H. (2005). Multicellularity, stem cells, and the neoblasts of the planarian *Schmidtea mediterranea*. *Experimental Cell Research, 306*, 299–308. https://doi.org/10.1016/j.yexcr.2005.03.020.

Sarnat, H. B., & Netsky, M. G. (2002). When does a ganglion become a brain? Evolutionary origin of the central nervous system. *Seminars in Pediatric Neurology, 9*, 240–253. https://doi.org/10.1053/spen.2002.32502.

Schürmann, W., & Peter, R. (2001). Planarian cell culture: A comparative review of methods and an improved protocol for primary cultures of neoblasts. *The Belgian Journal of Zoology, 131*(Suppl. 1), 123–130.

Scimone, M. L., Kravarik, K. M., Lapan, S. W., & Reddien, P. W. (2014). Neoblast specialization in regeneration of the planarian *Schmidtea mediterranea*. *Stem Cell Reports, 3*(2), 339–352. https://doi.org/10.1016/j.stemcr.2014.06.001.

Seilern-Aspang, F., & Kratochwil, K. (1965). Relation between regeneration and tumor growth. In V. Kiortsis & H. Trampusch (Eds.), *Regeneration in animals and related problems* (pp. 452–473). Amsterdam: North Holland.

Sikes, J. M., & Newmark, P. A. (2013). Restoration of anterior regeneration in a planarian with limited regenerative ability. *Nature, 500*(7460), 77–80. https://doi.org/10.1038/nature12403.

Solana, J. (2013). Closing the circle of germline and stem cells: The Primordial Stem Cell hypothesis. *EvoDevo*. https://doi.org/10.1186/2041-9139-4-2.

Strassmann, J. E., & Queller, D. C. (2010). The social organism: Congresses, parties and committees. *Evolution, 64*(3), 605–616. https://doi.org/10.1111/j.1558-5646.2009.00929.x.

Stückerman, T., Cleland, J. P., Werner, S., Vu, H. T.-K., Bayersdorf, R., Liu, S.-Y., Friedrich, B., Jülicher, F., & Rink, J. C. (2017). Antagonistic self-organizing patterning systems control maintenance and regeneration of the anteroposterior axis in planarians.

Developmental Cell, 40, 248–263. https://doi.org/10.1016/j.devcel.2016.12.024.

Szathmáry, E. (2015). Toward major evolutionary transitions theory 2.0. *Proceedings of the National Academy of Sciences of the United States of America, 112*(33), 10104–10111. https://doi.org/10.1073/pnas.1421398112.

Tiozzo, S., & Copley, R. R. (2016). Reconsidering regeneration in metazoans: An evo-devo approach. *Frontiers in Ecology and Evolution, 3*, 67. https://doi.org/10.3389/fevo.2015.00067.

Umesono, Y., & Agata, K. (2009). Evolution and regeneration of the planarian central nervous system. *Development, Growth & Differentiation, 51*, 185–195. https://doi.org/10.1111/j.1440-169X.2009.01099.x.

van Wolfswinkle, J. C., Wagner, D. E., & Reddien, P. W. (2014). Single-cell analysis reveals functionally distinct classes within the planarian stem cell compartment. *Cell Stem Cell, 15*, 326–339. https://doi.org/10.1016/j.stem.2014.06.007.

Wagner, D. E., Wang, I. E., & Reddien, P. W. (2011). Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. *Science, 332*(6031), 811–816. https://doi.org/10.1126/science.1203983.

West, S. A., Fisher, R. M., Gardner, A., & Kiers, E. T. (2015). Major evolutionary transitions in individuality. *Proc. Natl. Acad. Sci. USA* 112(33), 10112–10119. https://doi.org/10.1073/pnas.1421402112.

West, S. A., & Kiers, E. T. (2009). Evolution: What is an organism? *Current Biology, 19*(23), R1080-R1082. https://doi.org/10.1016/j.cub.2009.10.048.

Zattara, E. E. (2015). Transplants in annelids, nemerteans and planarians: A tool for embryology, immunology, endocrinology and regeneration research. *Invertebrate Survival Journal, 12*, 247–263.

Zattara, E. E., & Bely, A. E. (2016). Phylogenetic distribution of regeneration and asexual reproduction in Annelida: Regeneration is ancestral and fission evolves in regenerative clades. *Invert. Biol., 135*(4), 400–414. https://doi.org/10.1111/ivb.12151.

Zhu, S. J., & Pearson, B. J. (2016). Neo)blast from the past: New insights into planarian stem cell lineages. *Curr. Opin. Genet. Devel., 40*, 74–80. https://doi.org/10.1016/j.gde.2016.06.007.