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REVIEW

# Amphimixis and the individual in evolving populations: does Weismann's Doctrine apply to all, most or a few organisms?

**Karl J. Niklas · Ulrich Kutschera**

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**Abstract** The German biologist August Weismann (1834–1914) proposed that amphimixis (sexual reproduction) creates variability for natural selection to act upon, and hence he became one of the founders of the Neo-Darwinian theory of biological evolution. He is perhaps best known for what is called "Weismann´s Doctrine" or "Weismann´s Barrier" (i.e. the irreversible separation of somatic and germ cell functionalities early during ontogeny in multicellular organisms). This concept provided an unassailable argument against "soft inheritance" *sensu* Lamarck and informed subsequent theorists that the only "individual" in the context of evolution is the mature, reproductive organism. Herein, we review representative model organisms whose embryology conforms to Weismann´s Doctrine (e.g. flies and mammals) and those that do not (e.g. freshwater hydroids and plants). Based on this survey and the Five Kingdoms of Life scheme, we point out that most species (notably bacteria, fungi, protists and plants) are "non-Weismannian" in ways that make a canonical definition of the "individual" problematic if not impossible. We also review critical life history functional traits that allow us to create a matrix of all theoretically conceivable life cycles (for eukaryotic algae, embryophytes, fungi and animals), which permits us to establish where in this scheme Weismann's Doctrine holds true and where it does not. In addition, we argue that bacteria, the dominant organisms of the biosphere, exist in super-cellular biofilms but rarely as single (planktonic) microbes. Our analysis attempts to show that competition among genomic variants in cell lineages played a critical part in the evolution of multicellularity and life cycle diversity. This feature was largely ignored during the formulation of the synthetic theory of biological evolution and its subsequent elaborations.

**Keywords** Evolutionary theory · Expanded synthesis · Individuality · Life cycle evolution · Multicellularity · Multilevel selection theory

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## Introduction

Like the British naturalists Charles Darwin (1809–1882) and Alfred Russel Wallace (1823–1913), the German zoologist and evolutionary biologist August Weismann (1834–1914) was, at an early age, a curiosity-driven collector of beetles, butterflies and plants. However, unlike his more famous colleagues, Weismann took over the position of a professorship at the Albert Ludwig University of Freiburg im Breisgau (Fig. 1). Using the light microscopic techniques available in his Institute, Weismann studied the development of insects and small crustaceans (ostracods and daphinids). In subsequent studies, he examined hydrozoans with a focus on elucidating the fate of germ cells. On these and related topics, he published numerous research papers and monographs (Gaupp 1917). However, Weismann is perhaps best known as an outstanding theorist who has been described by Ernst Mayr (1904–2005) as "one of the greatest biologists of all times" (Mayr 1982, p . 698), ranked second in importance only to Darwin.

Today, Weismann´s main theoretical contributions to biology are considered to be the role of sexual reproduction (amphimixis) in animals and the germ plasm theory, which collectively removed any doubt that the inheritance of acquired characteristics, as proposed by Jean-Baptiste Lamarck

**Fig. 1** Photograph of August Weismann (1834–1914), at the age of ca. 70 years, when he was full Professor and Director of the Institute of Zoology at the University of Freiburg im Breisgau, Germany

(1744–1829), is impossible. Weismann embraced Darwin's theory of evolution and referred, in this context, to the equally important work of Alfred Russel Wallace (Weismann 1886, 1889). However, above all of his contemporaries, he realised that no debate about its validity could be resolved without a comprehensive, verifiable theory of inheritance. In 1876, Weismann provided such a theory in which he concluded that inheritance is the result of molecular movements, an idea he later abandoned. During the early 1870s, Weismann maintained that external conditions could influence inherited characteristics, as proposed by Lamarck (1809) and Darwin (1859, 1872) (but not by Wallace 1889, see Kutschera and Hossfeld 2013). Later, he rejected this concept (Weismann 1889), but only in part as he continued to believe that external factors could alter the heritable materials in organisms (Winther 2001). Ironically, this feature of Weismann's theory was either ignored or swept aside by the majority of his subsequent adherents.

Ten years ago, we summarised the achievements of August Weismann with respect to the role his discoveries played in the formulation of the Neo-Darwinian theory (Kutschera and Niklas 2004). In this article, which marks the 100th anniversary of Weismann's death (5 November 1914), we re-describe his concepts and theories on inheritance and natural selection and discuss the role played by the individual in evolving populations in terms of a broad phyletic perspective of what the "individual" is and the recent claim of the existence of a "germ line" in plants (Berger and Twell 2011; Whipple 2012). The goals of the following sections are fourfold: (1) to review the evidence supporting Weismann's Doctrine, (2) to show that this principle applies to comparatively few evolving lineages (all of which are late-divergent and persistent groups of macro-organisms, essentially the metazoa and embryophytes), (3) to provide evidence showing that germ line cells are not sequestered early in ontogeny for a large number of different kinds of organisms, (4) to show that this divergence from Weismann's Doctrine requires different definitions of "individuality" and (5) to construct and review a constellation of life history traits that identify a matrix of all theoretically possible life cycles that help to establish where and when Weismann's Doctrine holds true and where it does not.

## Weismann's mouse tail experiments

In 1875, Weismann began to realise that "soft inheritance" *sensu* Lamarck-Darwin was an untenable proposition. Between 1875 and 1880, he experimented with populations of mice (*Mus musculus*) by cutting off their tails and reported that "901 young were produced by five generations of artificially mutilated parents and yet there was not a single example of a rudimentary tail or any other abnormality of the organ" (Weismann 1889). In a subsequent publication, Weismann (1892) reported that even in the 19th generation no inheritable "cutting effect" occurred (Fig. 2). Based on these experiments and careful embryological observations, Weismann proposed a vastly different theory of inheritance in 1883 and in 1885

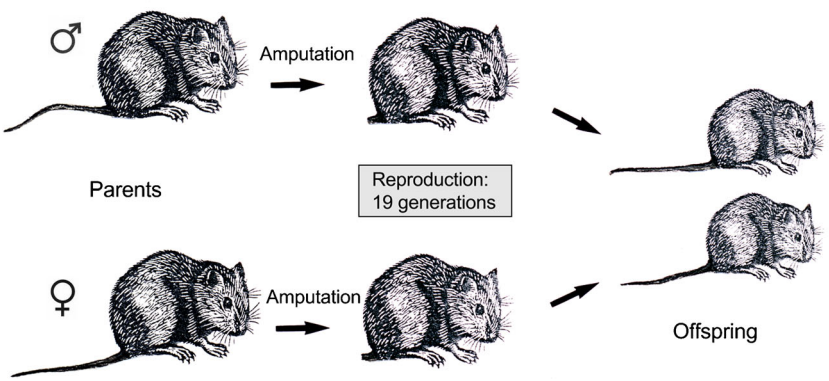


**Fig. 2** Schematic summary of Weismann's mouse-tail experiments, which spanned 19 generations and produced 901 young. As a result of these studies, on the species *Mus musculus*, he concluded that mutilations and other modifications of the soma are not heritable, i.e. Lamarck's soft inheritance is not correct

(Winther 2001). This extensively revised theory advanced three critical conceptualizations. Firstly, Weismann concluded that the genetic material is chemical in nature and not the result of molecular movement. Secondly, he reasoned that this material is contained in the nucleus (and borne on what are known today as chromosomes). Finally, he argued that somatic cellular terminal differentiation was the result of an irreversible dissection of the heritable chemistry of the "germ plasm" such that the environmental effects on somatic cells could have no possibility of being passed on to the next generation. Weismann argued that the heritable chemistry of an organism's parents was responsible for its phenotypic traits, although he remained an externalist regarding the causes of variation, i.e. external conditions acting on early development could change heritable materials (see Winther 2001).

The postulate that somatic cellular differentiation is terminal, inspired Weismann (1889) to consider further how the chemical nature of heredity affected ontogeny and how the germ and soma cell lines become separated during embryogenesis. Weismann assumed that ontogenetic changes had to reflect how the total genetic material in the fertilised egg is progressively dissected into smaller and smaller groups of different kinds of particles. This reasoning led Weismann to propose a very complex hierarchical system of molecules (and their collectives) that he believed controlled specific phenotypic features each of which results from a specific kind of particle (he called "biophores"). During development and cell division, Weismann proposed that each cell receives different kinds and numbers of "biophores", which resulted in cell differentiation (Weismann 1892). Most importantly, the zoologist argued that "amphimixis", i.e. the mixing up of heritable material from the female and male gametes (eggs and sperm) creates variable offspring in the next generation. Accordingly, Weismann (1892, 1913) proposed that sexual reproduction is the cause of variability among progeny, which is a necessary precondition for evolutionary changes via natural selection in ever-changing environments (Burt 2000; Kutschera and Niklas 2004; Bell 2008; Niklas 2014a).

Weismann's revised theory of inheritance was quickly criticised not only by neo-Lamarckians but also by "old-school" Darwinians who remained wedded to the idea that the effects of use and disuse are heritable and who saw Weismann's work as too narrow in its approach (Levit and Hoßfeld 2006). Botanists also criticised Weismann's theories because they were well aware that many plants rely on vegetative propagation and possess the capacity for somatic embryogenesis. Contending theories about inheritance were abundant as well, and some were remarkably close to current paradigms. For example, the Austrian botanist Gottlieb Haberlandt (1854–1945) demonstrated that "all plant cells are able to give rise to a complete plant" (Haberlandt 1904) and nearly anticipated the existence of mRNA when he proposed that the nucleus is the source of specific molecules that regulate the activities of the cytoplasm, molecules that Hugo de Vries (1848–1935) argued were enzymes (de Vries 1901). Indeed, particularly effective arguments were mustered against Weismann's "dissection" theory by the German zoologist Oscar Hertwig (1849–1922) and the German philosopher and biologist Hanns Driesch (1869–1914) (Hertwig 1894; Driesch 1899). Finally, many of Weismann's critics were aware of the work of the German embryologist Wilhelm Roux (1850–1924) who argued convincingly that the process of mitosis makes no sense unless the cellular machinery and heritable materials are partitioned equally between derivative cells (Roux 1883). Over time, the debates stimulated by Weismann's theory drifted away from how heritable materials were transmitted and more toward concepts concerning the mechanics of development.

## Weismann's Doctrine and its implications

The proposition that cellular differentiation denies somatic cells the opportunity to contribute heritable information to the next generation is now called "Weismann's Doctrine" or "Weismann's Barrier" (Fig. 3). This concept played an important role in the formulation of the modern synthesis during the early and mid-parts of the twentieth century, because it helped to establish the organism as the indivisible unit of biological organisation. This axiom was entirely consistent with the experimental evidence brought forth by the founders of the modern synthesis all of whom worked on multicellular organisms for which Weismann's concept of the individual was fully realised (e.g. species representing late-divergent animal lineages, such as fruit flies, hamsters and humans) (Kutschera and Niklas 2004; Kutschera 2009a, b). Indeed, as noted by Leo Buss: "… acceptance of the modern synthesis stands as compelling testimony to the fact that evolution has manifestly favoured ontogenies in which the Weismannian ideal is approximated" (Buss 1987, p. 4). This bias in the selection of model experimental organisms had a curiously negative effect regarding the role embryology played during the formulation of the synthetic theory. The selection of a biological model for any purpose can have a profound effect on the conclusions drawn from that model (see Bolker 2014). If variations arising in somatic cells during the course of ontogeny play no part in heredity, the dynamics of the soma are largely irrelevant to evolutionary biology and theory. This perspective was famously captured by a classic diagram of Weismann's doctrine appearing in *Life, An Introduction to Biology*, an extremely influential biology textbook authored by Simpson et al. (1957). A modified version of this scheme is depicted in Fig. 3, and a simplified version of this image was published by Buss (1987). Obviously, Weismann (1892, 1913) had mammals, such as his lab mice, in mind (see Fig. 2) when he first described this novel theory of inheritance.

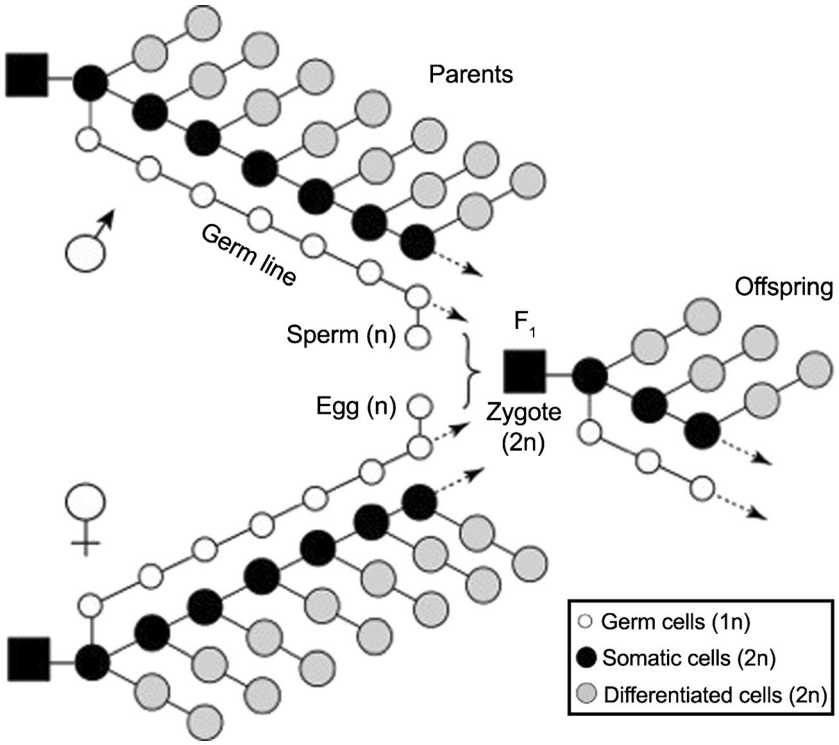


**Fig. 3** Scheme illustrating August Weismann's concept of the separation of the germ line from the soma in multicellular organisms (animals, such as fruit flies, mice and humans). Note that amphimixis (sexual reproduction) creates variable offspring, whereas somatic mutations do not contribute to the next generation, i.e. the germ line of cells is sequestered early in ontogeny

## Five Kingdoms and the individual

We stated earlier that Weismann's Doctrine played an important role in the formulation of the synthetic theory because it established the organism as the indivisible unit of biological organisation, and thus the primary focus or "target" of natural selection. Although it was clear to them that evolutionary theory requires a holistic perspective, one that emphasizes relationships among different hierarchical levels (see Mayr 1982, pp. 66–67), the assumption that the organism is the individual is implicit in most modern biology textbooks, wherein it is common to read that an individual organism is defined on a "one genome-in-one-body" concept. This classical "animals–plants perspective" is in conflict with the more sophisticated "Five Kingdoms of Life" scheme that, in addition to the Animalia and Plantae, has incorporated three additional Kingdoms (Fungi, Protoctista and Bacteria). These lesser popular eukaryotic and prokaryotic organisms are, based on their combined protoplasmic biomass, the dominant organisms of the biosphere (Kutschera and Niklas 2004). Nevertheless, the extent to which the individual and the organism are one in the same body is not always clear, particularly when dealing with colonial organisms, or living beings that rely in part or entirely on asexual reproduction. For these kinds of organisms, the unit of selection can be a clone of genetically identical individuals. Indeed, in some cases it may be legitimate to conceive of an entire colony as an individual, which represents the "target" of natural selection. Surprisingly, there has been little empirical work devoted to answering how the "individual" can or should be defined, because it is contextually dependent. If the individual is defined as the unit of natural selection, it can reside at the level of individual genes, gene networks, entire genomes, clones or even entire species (Wilson 1999; Wilson and Barker 2013).

In the context of what follows, we conceive of an individual as any biological entity that has undergone an *alignment-of-fitness* and an *export-of-fitness* among its constitutive parts (*sensu* Folse and Roughgarden 2010). These two criteria and their role in multilevel selection theory are described in greater detail below.

## Evidence supporting Weismann's Doctrine

In the context of contemporary biological theory, Weismann's Doctrine (i.e. the principle that heritable information is only transferred from the genome to the soma and not vice versa) retains three important elements: (1) early in ontogeny, cells that participate in reproduction are sequestered from somatic cells, (2) the experiential "history" of somatic cells therefore cannot contribute to the phenotype of the next generation and (3) the "individual" defined in the context of Darwinian evolution is the reproductively viable adult organism (and not any of its constituent cells, tissues or organs, as earlier workers have suggested).

Arguably perhaps, the classic example of the "ideal Weismannian organism" is the fruit fly *Drosophila melanogaster*, which was an important model organism used

to develop and refine Mendelian genetics and thus formulate the synthetic theory. Theodosius Dobzhansky (1900–1975), one of the major architects of this concept in the 1940s, analysed the biology of *Drosophila* species and drew important evolutionary conclusions, based on his experimental findings (Kutschera and Niklas 2004; Kutschera 2009a, b) (Fig. 4).

We described the fruit fly as an "ideal organism" because its embryology is remarkably consistent with how Weismann conceived of the sequestration of the germ plasm from the soma. To be specific, shortly after the fusion of sperm and egg cells (i.e. amphimixis), the zygotic nucleus undergoes 13 rapid free-nuclear divisions to produce a coenocyte containing 8,192 nuclei. At the closure of the 13th division cycle, cellularization occurs to produce a multicellular embryo consisting of two distinct regions, the blastoderm consisting of approximately 6,000 somatic cells and a congeries of roughly 2,160 germ track pole cells. At this juncture, somatic and germ line nuclei are differentially sorted permanently. Thus, the only possible opportunity that genetically variant nuclei could enter the germ cell line is during the first thirteen cycles of nuclear divisions and it is precisely during this time that the material genome exerts virtually unfettered control. Evidence for this claim comes from a number of experimental studies reporting exceptionally low rates of mRNA synthesis before the blastoderm is formed (Fausto-Sterling et al. 1974; Gilbert 2006).

Similarly, the embryological fate of cells during the early development of the nematode *Parascaris aequorum* (round worm) is equally illustrative, as the fate of cells is established

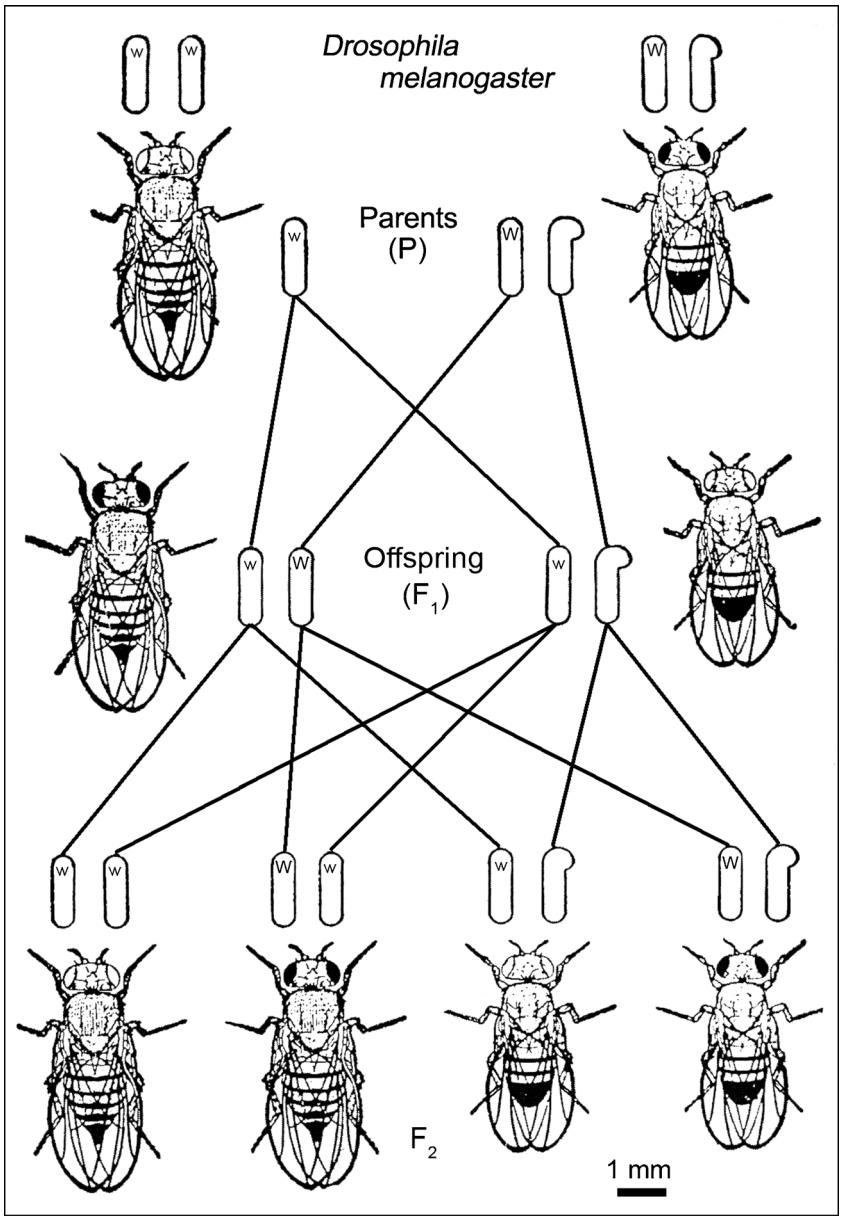


**Fig. 4** The common fruit fly (*Drosophila melanogaster*). Wild-type (chromosomes denoted with w) and mutated individuals (chromosomes lacking w) served as important model organisms for the elucidation of the germ line in developing insects as well as helped to re-establish and expand on Mendelian genetics

and under strict material control during the first four cleavage divisions. The first such division results in an uncommitted cell and the first somablast, which is terminally destined to form the primary ectoderm. The uncommitted cell then divides to produce another undifferentiated cell and the secondary somablast, which is programmed to develop into the endoderm and the primary mesoderm. Two subsequent cell division cycles produce all somatic cell lineages with the exception of the primordial germ cell track. Once again, experimental evidence reveals that all of these cell division cycles are under material control until the formation of the germ cells (Gilbert 2006). Similar results have been obtained in studies on the bacteriovorous nematode (round worm) *Caenorhabditis elegans*, one of the major model organisms for the study of development in invertebrates (Fig. 5).

Freshwater crustaceans of the genus *Daphnia* are organisms that were studied by Weismann (1908, 1913) in detail. Species such as *Daphnia pulex* have a life cycle alternating between asexual (parthenogenetic) and sexual reproduction, which has been called a "cyclical parthenogenesis" mode of development (Kutschera 2010). Throughout the majority of the growing season, adults function as females producing diploid eggs that develop into functionally female adults. However, toward the end of the growing season, females produce tough diploid eggs, which are called "resting" or "winter" eggs that develop typically into females. However, some of the resting eggs also develop into males that can fertilise eggs that develop into females, which subsequently produce diploid eggs (Lynch et al. 2008). Some species never produce males and thus only reproduce parthenogenetically (Fig. 6). Using a zinc-finger-containing VASA marker, Sagawa et al. (2005) have shown germ cell primordia are sequestered early during the cleavage stage in embryology in parthenogenetic and resting eggs. It is worth noting that parthenogeneticity and other forms of asexual reproduction increase the fitness of an individual genome by virtue of multiplying the individuals carrying it, as suspected by Weismann, when he wrote: "As soon, however, as parthenogenesis becomes advantageous to the species … it will not only be the case that colonies which produce the fewest males will gain advantage, but within the limits of the colony itself, those [colonies of] females will gain advantage which produce eggs that can develop without fertilisation" (Weismann 1889, p. 326).

## Freshwater algae and mammals

Planktonic organisms, such as unicellular algae, evolved via primary and secondary (paleo) endosymbiotic events. These photosynthetic microorganisms, which are of considerable ecological importance, were largely ignored by Darwin (1859, 1872) and Wallace (1889), due to the limited knowledge about the biology of these eukaryotic microbes (Kutschera and Niklas 2005, 2008). It is obvious that no germ line-soma differentiation occurs during the ontogenetic development of these species, which are major components of the marine phytoplankton.

We can turn to an entirely independent evolutionary experiment with multicellularity by drawing attention to the Volvocales, a lineage of green algae that figured prominently in Weismann's arsenal of examples of germ-soma separation (Weismann 1889, 1913). Among some of the early-divergent volvocalean taxa, *Gonium* and *Pandorina* provide examples of multicellular organisms evidencing no cellular differentiation. Each cell is totipotent and capable of producing new individuals. In contrast, species belonging to the genus *Volvox* manifest somatic and germ cell lines, the former cells bear flagella. Of the several thousands of cells, only a few cells in *Volvox* are capable of either asexual or sexual reproduction.

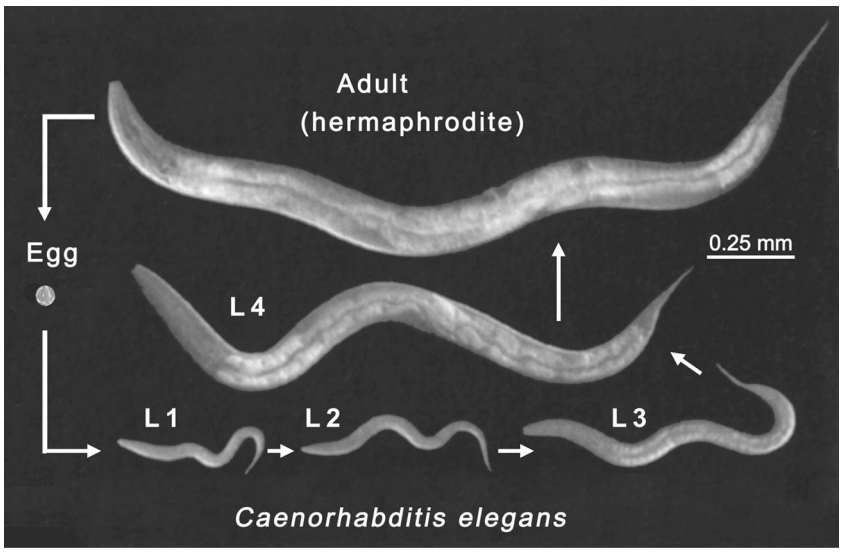


**Fig. 5** The bacteria-eating round worm *Caenorhabditis elegans*, a model system for the study of development and germ line vs. soma differentiation in invertebrates (adapted from Kutschera 2010)

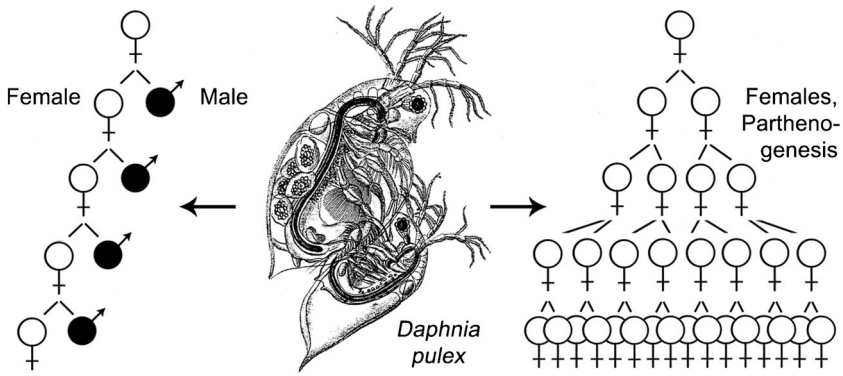


**Fig. 6** The common water crustacean *Daphnia pulex*, a freshwater invertebrate that can switch between parthenogenetic (asexual) and biparental (sexual) reproduction, respectively, depending on environmental conditions (adapted from Kutschera 2010)

Mutants of *Volvox* are reported some of which have lost the capacity for cellular differentiation. In these mutants, every cell is capable of functioning as a germ cell. These mutants illustrate the importance of cellular differentiation in the transition from a "non-Weismannian" organism (in which the distinction between soma-germ cell lines is lacking) to a "Weismannian" organism (in which cell differentiation establishes a distinction between the two) (Gilbert 2006).

Although it is generally true that a differentiated cell is denied the ability to contribute to inheritance, the de-differentiation of cells is not an uncommon phenomenon, as demonstrated by conversion of choanocytes into sperm cells in the sponge *Hippospongia* and the formation of gonadal cells from parientopleural cells in some annelids such as *Lumbricillus* (Gilbert 2006). Nevertheless, the separation of the germ from the soma cell track early in ontogeny occurs in organisms as diverse as rotifers, nematodes, ctenophorians, chaetognathians and chordates, such as the classical laboratory mouse (*M. musculus*) (Fig. 3). It also occurs in some molluscans, annelids and a number of other groups that adhere to Weismann's Doctrine (Bell 1982; Buss 1983, 1987), i.e. genetic variants of somatic cells *cannot* contribute heritable information to the next generation. Accordingly, in these species of "lower" and "higher" animals (i.e. insects and mammals), the "individual" is invariably the reproductive, sexually mature adult organism, as proposed by Weismann (1886, 1889, 1892, 1913).

## Evidence incompatible with Weismann's Doctrine

Noticeably absent from the foregoing list of organisms are the majority of species in all of the algal clades, the land plants (embryophytes), the fungi and a diverse spectrum of species belonging to the Placozoa, Cnidaria, Porifera and myxomycetes (Buss 1983, 1987; Hoppe and Kutschera 2010), all of which manifest somatic embryogenesis. Ironically, a model "non-Weismannian" organism was used by August Weismann to develop his theory of inheritance, the simple freshwater hydroid *Hydra viridis* (Fig. 7). Here, we briefly review the embryology of this hydroid and compare it to another model "non-Weismannian" organism, the common dandelion (*Taraxacum officinale*), a representative member of a monophyletic lineage, the land plants (Niklas and Kutschera 2009, 2010).

In a mature *H. viridis*, immediately following amphimixis (fusion of gametes), the zygote divides to give rise to two populations of cells, the somatic and the amoeboid interstitial cells. The latter are totipotent in the sense that any cell in this group can give rise to any somatic cell type. Among the somatic cells, some individuals are capable of additional division, whereas others are not. Those that belong to the latter

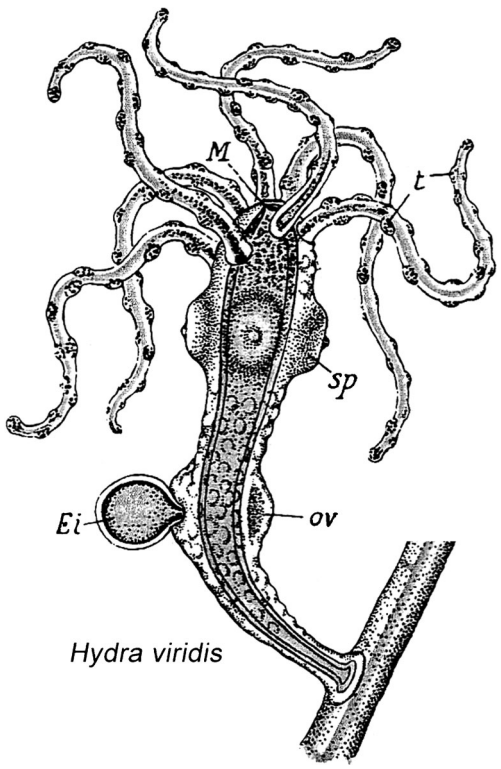


**Fig. 7** The freshwater hydroid *Hydra viridis*, an organisms used by August Weismann for the study of the development of germ line vs. somatic cells. Subsequent studies revealed that no clear separation of a germ line occurs during the ontogeny and subsequent reproductive biology of this ancient metazoan (adapted from Weismann 1913)

category are replaced by interstitial cells, which a botanist might easily call "meristematic cells". Asexual reproduction is the result of the movement of totipotent interstitial cells and somatic cells into a lateral bud that emerges from the side of the adult polyp. The bud can detach and carry on an independent existence. Interstitial cells also have the ability to form gametes, particularly when local environmental conditions deteriorate (Fig. 7). Thus, these cells can have one or more of three different fates: (1) they may give rise to somatic cells, (2) they can meiotically divide to form gametes and (3) they can remain meristematic to give rise to gametes or "vegetative" somatic cells (Gilbert 2006). The development of *Hydra* is extremely "non-Weismannian" in virtually every respect. In passing, it is worth noting that recent studies on the regulation of meiosis in *Hydra* indicate that sexual reproduction (amphimixis) evolved only once within the Kingdom Animalia more than 500 million years ago (Fraune et al. 2012). Analogues of this mode of development abound in the myxomycetes, red algae, stramenopiles, fungi and land plants (Buss 1987; Hoppe and Kutschera 2010).

Another example of a non-Weismannian organism is the common dandelion (*Taraxicum officinale*), whose reproductive structures (flowers) are produced by shoot apical meristems that can continue to produce derivative cells that can take on a somatic or a germ cell line function (Fig. 8). Among many flowering plant species, floral developmental results in carpels bearing ovules in which sporogenesis and gametogenesis give rise to haploid egg-producing plants (megagametophytes) that are subsequently fertilised by haploid sperm-producing plants (microgametophytes). Even in this well-orchestrated sequence of events, somatic variant cells tracing their origins back to the shoot apical meristem have some probability of contributing to the genetic composition of the next generation. This possibility is augmented by the fact that many if not most dandelion populations consist of triploid individuals that produce seeds parthenocarpically owing to the inability to produce viable egg or sperm cells by means of normal meiotic cell division. In these individuals, a triploid cell in the nucellus takes on the function of a zygote and develops into a triploid embryo.

Parthenocarpy is a common phenomenon in many flowering plant families, as for example the Crassulaceae members of which produce plantlets on the margins of their leaves (e.g. "the Mother of Thousands" *Kalanchoë daigremontiana*). Another example is seen in the crassulacean *Bryophyllum tubiflorum* (Fig. 9a). As in many other species, somatic genomic changes in the cells of *B. rubiflorum* can be perpetuated by means of asexual reproduction. These and many more examples of plant, fungal and myxomycete life cycles show that genetic variants of somatic cells *can and do* contribute to the inheritance of the next generation. Although some botanists have argued that a germ line exists in the flowering plants, analogous to that in animals (Berger and Twell 2011; Whipple 2012), the evidence drawn from

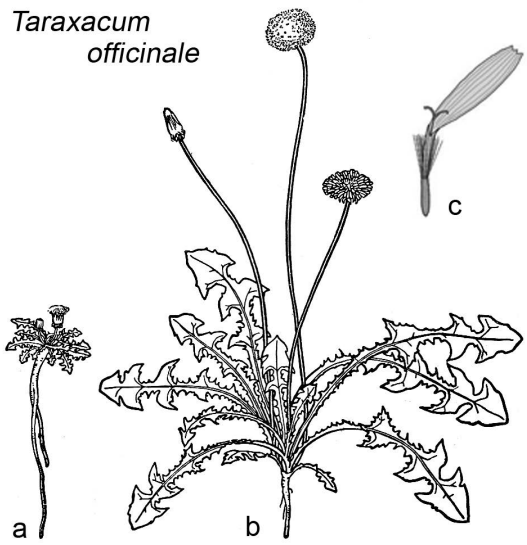


**Fig. 8** Juvenile (**a**) and mature sporophyte (**b**) of the dandelion (*Taraxacum officinale*) and a single ray flower (**c**). In this common species, embryos can develop in flowers from somatic as well as germ line cells

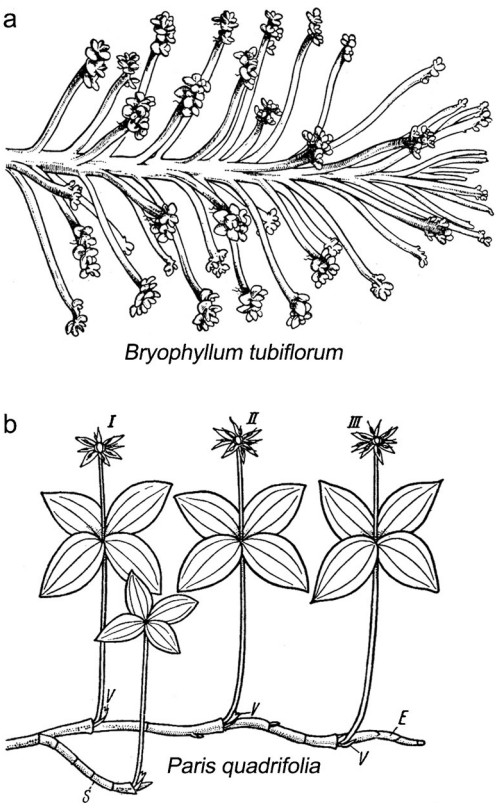


**Fig. 9** Vegetative reproduction in embryophytes. The Chandelier plant (*Bryophyllum tubiflorum*) from Southern Madagascar, with daughter plantlets (**a**). The Herb Paris (*Paris quadrifolia*) from Europe, consisting of a large rhizome that can fragment and clone new plants and single shoots that develop flowers for sexual reproduction (**b**)

comparative plant reproductive biology does not support the existence of Weismann's Barrier in the angiosperms. This major conclusion is illustrated by the scheme shown in Fig. 10. Under any circumstances, many species of flowering plants, such as duckweeds (Kutschera and Niklas 2014) or the Herb Paris (*Paris quadrifolia*) propagate vegetatively via fragmentation and/or rhizomatous growth, such that "the individual plant" is a clone (Fig. 9b).

In summary, *contra* Weismann's Doctrine, the conceptualization in the synthetic theory of the adult multicellular organism as an "indivisible unit" of selection is not applicable to a vast number of eukaryotic organisms. Indeed, among the majority of multicellular species, the individual is not invariably the single organism, but rather, in many cases, different cell lineages competing among one another for access to germ cell functionalities (Buss 1983, 1987).

## Individuality and the evolution of multicellular organisms

Even a brief review of algal, plant, fungal and animal embryology reveals a spectrum of developmental patterns whose extremes conform with Weismann's Doctrine, or sharply deviate from it, depending on whether we examine representative species drawn from early-divergent or more derived evolutionary lineages. Our objective is not to polarise these extremes but rather to draw attention to the impossibility of standardising what is meant by the "individual" in ways that would make such a definition equally applicable to all lineages (see Herron et al. 2013). As noted earlier, for some eukaryotic organisms, the "individual" may be the entire living being (animals, such as humans, mice and flies) (Figs. 1, 2 and 4), whereas for others it may be cells, cell lineages competing for reproductive success or vegetatively propagating clones (Fig. 9b). This speculation is consistent with the multilevel selection theory and the evolution of multicellularity (Damuth and Heisler 1988; Michod and Nedelcu 2003; Niklas and Newman 2013; Niklas et al. 2013; Niklas 2014b).

Multilevel selection theory identifies two evolutionary phases for the phylogenetic development of a multicellular organism—*alignment-of-fitness* phase (denoted as MLS1) in which genetic similarity among adjoining cells prevents cell–cell conflict, and *an export-of-fitness* phase (denoted as MLS2), in which cells become interdependent and collaborate in a sustained physiological and reproductive effort (for a general review, see Folse and Roughgarden 2010). Phyletic analyses of lineages in which obligate multicellularity has evolved are consistent with this two-step model. MLS1 is comparatively easily achieved by a "unicellular bottleneck", i.e. the appearance of unicellular uni-nucleate cells somewhere in an organism's life cycle, e.g. a spore, zygote or uni-nucleate asexual propagule (see Niklas and Newman 2013). This bottleneck establishes an initially genetic homogeneity among subsequently formed cells (or, more precisely, among nuclei). Nevertheless, subsequent genetic heterogeneity can develop in a variety of ways.

Indeed, competitive–cooperative interactions have shaped form–function relationships even at the simple molecular level

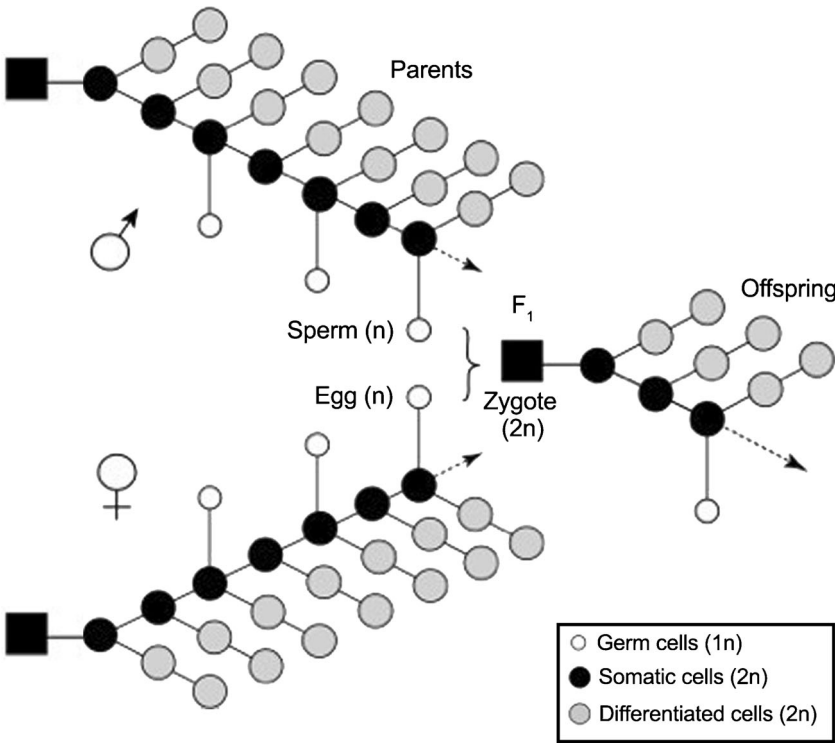


**Fig. 10** Scheme illustrating the germ line concept in land plants and many algal lineages (compare with Fig. 3), in which somatic cells can give rise to germ cells as well as other somatic cells (capable of differentiating). As a result, somatic mutations have the potential to contribute to the genomic variation in the next generation (for convenience, this diagram illustrates biparental species but applies equally to hermaphroditic species)

(Foster 2011). Consider that many developmental processes employ lateral inhibition in which neighbouring cells or cell lineages differing in their genomic composition compete to adopt the same fate. For example, during gonad development in *C. elegans* (Fig. 5), cells compete to develop into either a terminally differentiated, or a ventral uterine precursor cell. Success is determined by the relative amounts of the LIN-12 receptor and its LAG-2 ligand (Greenwald 1998). In a similar manner, cells compete during the development of *D. melanogaster* wings (Fig. 4). Thus, the ubiquitous expression of Myc abolishes cell competition and wings become larger than normal, whereas the addition of even a few wild-type cells results in renewed competition and brings wing size back to normal (de la Cova et al. 2004).

It is well known that mitosis does not always produce genetically identical derivative cells even in the absence of mutation or chromosomal aberrations. Preferential sister chromatid segregation occurs in plants, fungi and animals (Lark 1967; Lark et al. 1966; Rosenberger and Kessel 1968). For example, during the early development of female mammals (e.g., mice and humans), one of the two X chromosomes becomes silenced (Barr body or sex chromatin) and is faithfully perpetuated during subsequent cell divisions (Lyon 1961; Chow et al. 2005). Methylation patterns of cytosine in CpG doublets and other epigenetic changes provide additional avenues for the production of genetically different cell lineages, even in "ideal" Weismannian organisms (see Holliday and Grigg 1993).

It should be noted in this context that competition among nuclei occurs in fungi. For example, nuclear ratios of heterokaryons in the ascomycetes *Penicillium cyclopodium* and *Neurospora crassa* are reported to change, depending on environmental conditions, in ways that reflect the underlying fitness of the constituent homokaryons grown in isolation (Jinks 1952; Davis 1960). More recently, James et al. (2008) reported similar results for *Heterobasidion parviporum* and conclude that this basidiomycete violates the standard model for "individuality", as genetically different nuclei compete to form homokaryotic hyphae. However, as the previous examples show, an absence of conflict does not mean the lack of competition. Indeed, epigenetic mechanisms are likely essential to *maintain* multicellularity. In this context, we note that an important limitation to maintaining cooperation is dealing with "defectors", that is, cells or cell lineages that consume resources but fail to confer any benefit to the collective (Hamilton 1964), as for example animal neoplasms.

## Cheaters and the collective organism

A number of mechanisms have evolved to maintain cooperation and reduce the probability that defectors appear, e.g. the effects of group selection, direct and indirect reciprocity, network structure and tag-based donation schemes (Nowak 2006; Celiker and Gore 2013). However, to be successful, players are typically required to remember past events, or to possess some method of recognising cooperative from non-cooperative players. Epigenetic mechanisms as well as signalling pathways that connect metabolic status with nutrient availability or other environmental factors satisfy some of these requirements, e.g. the TOR signalling pathway. However, other tactics exist. Mathematical models show that resource limitations can cause the rules of a game to change in ways that foster cooperation among players with no memory and no recognition of one another (Requejo and Camacho 2013). Furthermore, zero-determinant models show that altruistic and generous strategies can sustain cooperation and reduce negative interactions (e.g. Stewart and Plotkin 2013).

Curiously, cheaters need not be inevitably deleterious. For example, mutant cells in the social amoeba (*Dictyostelium discoideum*) and in the mouse (*M. musculus*) are reported to cooperate in ways that conform to normal developmental patterns, i.e. they do not disrupt the functionality of the collective organism (Santorelli et al. 2008; Dejosez et al. 2013). Finally, it has been even speculated that cheaters may have functioned as ancient asexual propagules in some of the first proto-life cycles (Rainey and Kerr 2010).

Although cooperation and competition among genomically different cells and nuclei likely evolved along many different paths, the export-of-fitness phase to a truly multicellular organism required the emergence of an integrated entity capable of parenting a similar integrated phenotype with a heritable fitness. The critical difference between MLS1 and MLS2 is that the fitness of a congeries of cells is an additive function of the fitness of individual cells. By contrast, the fitness of a multicellular organism is non-additive (Damuth and Heisler 1988), i.e. the evolution of a multicellular organism requires a means to guarantee the heritability of fitness at the emergent level of the multicellular entity. In some multicellular organisms, MLS2 is accomplished by sequestering a germ cell line, whereas in other organisms the isolation of a germ cell line is not achieved (Dickson and Grant-Downton 2009). A germ-soma separation may be an indirect consequence of the necessity to compensate for the increasing costs of evolving a progressively larger body size (Solari et al. 2013), because the probability of compounding a genetic error or mutation increases as a function of the number of cell divisions required to achieve the size of a mature organism.

Small multicellular organisms have a lower probability of introducing errors into their reproductive cells because of the smaller number of division cycles required to achieve the body size of the reproductive adult, whereas progressively larger organisms escape "Muller's ratchet" (the inevitable accumulation of deleterious mutations), in some cases by sequestering cells in a germ line. Nevertheless, the probability that a rouge cell can take over a congeries of cooperating cells

decreases as the total number of cells increases as demonstrated mathematically by the formula

$$P_{fix} = \frac{1-\left(\frac{1-P_r}{P_r}\right)}{1-\left(\frac{1-P_r}{P_r}\right)^n},$$

where $P_{fix}$ is the probability a single rouge cell reaches fixation, $P_r$ is the probability that it will replace its normal counterparts, and $n$ is the total number of competing cells (Bozic and Nowak 2013). If we assign a rouge cell a slight advantage such that $P_r$=0.51, we see that $P_{fix}$=1.19 when $n$=5 but drops to $P_{fix}$=0.19 when $n$=15. This simple formula may provide some insights into one of the evolutionary drivers favouring multicellularity.

Plants and to a lesser extent fungi are less susceptible to the deleterious effects of mutations or "rogue cells" emerging over the course of their ontogeny by virtue of possessing rigid walls that preclude the migration of mutant nuclei. By contrast, animals evolved complex histochemical recognition systems that permit cell migration (while simultaneous restraining harmful nuclei from dominating during development). This may be the reason why, in many cases, germ line sequestration occurs early in their embryological development (Gilbert 2006).

## Evolution of life cycles

With the evolutionary appearance of simple multicellularity in each of the major eukaryotic clades, natural selection became focused on the collective individual as well as on its individual cell lineages (Niklas 2014b). The intensity and the foci of selection differed among the clades depending on the extent to which cell lineages cooperated or competed, and different constraints were imposed on selection depending on the ancestral functional traits charactering each clade, e.g. whether a rigid cell wall was ancestral and retained in late-divergent lineages. This in turn dictated the subsequent course of life cycle evolution. For example, as mentioned earlier, the early sequestration of a germ line in late-divergent animal lineages to cope with the ability of cells to move precluded asexual reproduction, whereas somatic embryogenesis, coupled with the presence of rigid cell walls permitted the evolution of life cycles with asexual and sexual reproductive phases.

Indeed, it is not difficult to construct a matrix of all theoretically possible life cycles using a comparative small number of life history functional traits (Niklas and Kutschera 2009). For example, a simple matrix can be constructed using four traits—the presence or absence of rigid cell walls, the type of life phase and its ploidy, the type of body plan, and the presence or absence of sexual and asexual reproductive phases. This matrix contains a total of 72 theoretically possible life cycles (Fig. 11). Eight of these combinations are biologically impossible because they combine "asexual reproduction" with an alternation of a diploid with a haploid individual. Some combinations describe the life cycles of many species, whereas others occur in only a few species or in none known to us. For example, most land plants (embryophytes) have life cycles described by "rigid cell walls"+"an alternation of generations"+"multicellular body plan"+"both asexual and sexual reproduction" (e.g. *Ribus* and *Equisetum*) (Niklas and Kutschera 2009, 2010). However, we are unaware of any organism with a life cycle described by "rigid cell walls"+"zygotic meiosis+"unicellular cellular body plan"+"sexual reproduction (only)", although many species have a life cycle consisting of "rigid cell walls"+"zygotic meiosis"+"unicellular body plan"+"asexual reproduction" (e.g. *Chlorella*).

We fully recognise that this matrix is a polite fiction because it neglects many other important functional traits, particularly the degree to which cells differentiate into functional types and if or when germ track cells are isolated during ontogeny. Nevertheless, the extent to which this matrix is occupied by species provides some insights into the evolution of life cycles when juxtaposed with the multilevel selection model for the alignment-of-fitness phase (MLS1) and the export-of-fitness phase (MLS2) discussed previously. This model posits a very specific transformation series in body plans (i.e. unicellular ⇒ colonial ⇒ simple multicellular ⇒ complex multicellular) that is empirically supported when each of the major algal clades is examined cladistically (see Niklas and Newman 2013), although an alternative route exists (i.e. coenocyte ⇒ simple multicellular ⇒ complex multicellular; see Niklas 2014b). Superimposition of either of these transformation series over the simple matrix of biologically possible life cycles indicates that "Weismannian organisms" occupy only a small portion of the matrix, and that the majority of plants and fungi evolved life cycles with asexual and sexual phases in which most nuclei had the possibility to contribute genomically to both the haploid and the diploid generation.

Space does not permit us to explore all relevant aspects of the evolution of life cycles. However, a treatment of the diphenic life cycle of the embryophytes serves as one particularly interesting example because it illustrates how the alternation of generations may have evolved as a consequence of competing maternal and paternal gene network expression patterns responding to an unpredictable or heterogeneous environmental conditions. It also provides a clue regarding how the body size of the diploid multicellular generation progressively increased during embryophyte evolution (see Niklas and Kutschera 2010; Niklas 2014b) as a consequence of genomic conflict (i.e. parent-specific gene expression

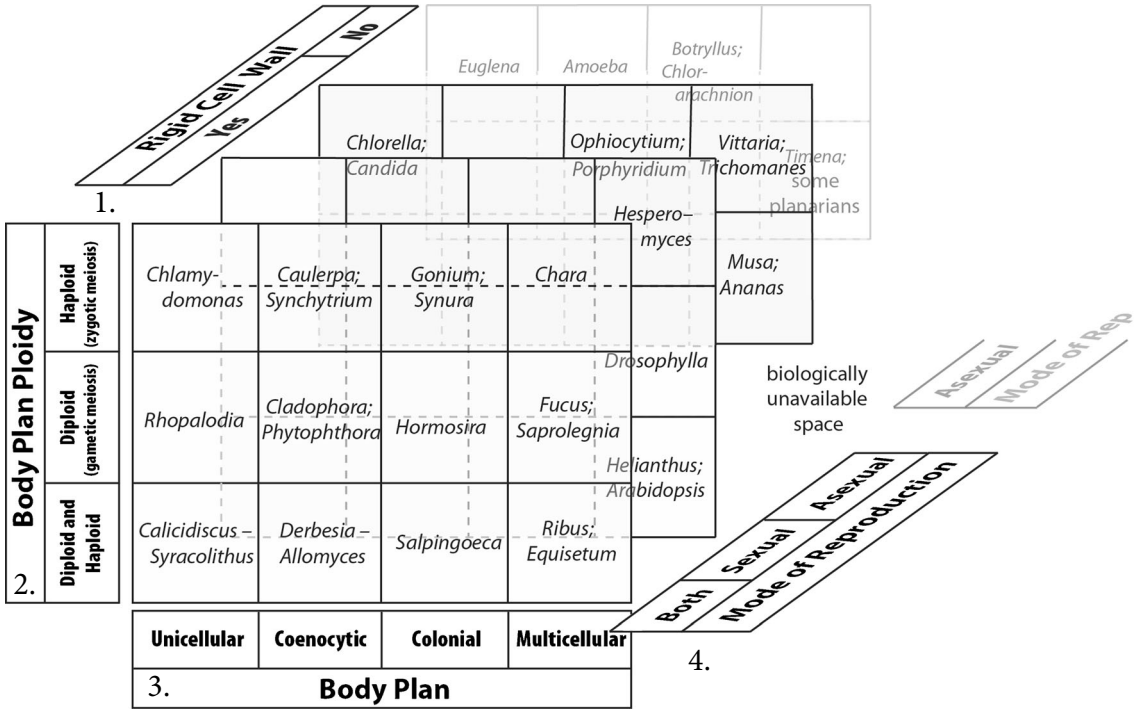


**Fig. 11** Schematic view of a theoretical matrix showing theoretically possible life cycles in different organisms based on four functional traits: (1) the presence or absence of a rigid cell wall, (2) the ploidy level of the body plan(s) in the life cycle, (3) the type of body plan (unicellular, coenocytic, colonial or multicellular) and (4) the mode(s) of reproduction (asexual, sexual or both). Seventy-two theoretical possibilities exist, but eight are biologically impossible (e.g. an organism possessing an alternation of generations that reproduces asexually exclusively). For clarity, two panels of the life cycle variants are not shown (i.e. those for organisms lacking rigid cell walls that can reproduce sexually or that can reproduce asexually and sexually). Representative taxa are included for some of the biologically possible permutations; the absence of a representative organism in some panels does not indicate the non-existence of such an organism, although some biologically possible permutations may not exist

patterns; see Trivers and Burt 1999). We note first that the ancestral life cycle to that of the land plants was probably a halobiontic-haploid one, in which the only multicellular organism functioned as a gametophyte as a result of zygotic meiosis (Niklas and Kutschera 2010). We note further that this life cycle likely involved matrotrophy and oogamy (both are pleisomorphic character states in the streptophyte clade; Graham and Wilcox 2000). Under these conditions, the paternal genome is predicted to gain a selective advantage if it behaves "selfishly" by inducing higher levels of nutrient provisioning for the zygotes it sires because this would favour its perpetuation in subsequent generations (by either increasing the number or the vigour of subsequently formed haploid zoospores). Indeed, it might also evolve a capacity to delay zygotic meiosis to increase the number of subsequently formed haploid cells, which would provide an adaptive advantage to the maternal as well as the paternal genome when fertilisation events are rare (Searles 1980; Haig and Wilczek 2006). If resources are limited, the maternal genome (which has an mRNA advantage by virtue of producing oogamous gametes) might respond to selectively abort zygotes (a form of post-fertilisation "mate-choice") and divert nutrients to only the most vigorous. It might also divert some of resources to vegetative growth or asexual reproduction, particularly in a species with zero paternal metabolic investment in the development of offspring (which occurs in dioicious species; see Williams 1975). In either case, genome conflict can provide a driver for the evolutionary shift from a haplobiontic-haploid life cycle to a diplobiontic life cycle in which the multicellular gametophyte still dominates (e.g. *Physcomitrella patens*). In turn, the evolutionary reduction in the size of the gametophyte generation over the course of embryophyte evolution may reflect "heterozygote advantage", i.e. the ability to mask deleterious recessive mutations while permitting the accumulation of adaptive recessive mutations (Sellis et al. 2011).

It must be noted that nothing in this scenario explains the evolution of an isomorphic biphasic life cycle in isogamous or anisogamous organisms, such as the multicellular green algae *Ulva lactuca*. For this and other reasons, it is fair to say that the evolution of life cycles and the intrinsic and extrinsic factors that allow them to persist are poorly understood. Table 1 provides some of the advantages and disadvantages of zygotic meiosis (haplobiontic-haploid life cycle), gametic meiosis (haplobiontic-diploid life cycle) and an alternation of diploid

**Table 1** Benefits and costs of life cycles with zygotic meiosis (haplobiontic-haploid life cycle), gametic meiosis (haplobiontic-diploid life cycle), and an alternation of diploid and haploid generations (diplobiontic life cycle) (see Fig. 11)

| Benefits | Costs |
|---|---|
| Haplobiontic-haploid life cycle | |
| Zygotic meiosis immediately purges deleterious mutations | Each zygote yields only four (or multiple thereof) genetically different products |
| Lower cost of DNA replication | |
| Rapid life cycle | Requires *r*-selection; meiosis is a slow process |
| Economises nutrient uptake (higher cell surface area to volume) | Constrains body sizes |
| Haplobiontic-diploid life cycle | |
| Buffers deleterious mutations | If alleles are not recessive, mutation load is twice the mutation rate |
| New genes can be maintained before they duplicate in tandem | Requires over dominance |
| Genetic variation in offspring is high | Cost of sex can be high |
| Diplobiontic life cycle | |
| Permits each generation to adapt to a different niche | Requires different niches and typically heteromorphism |
| Combines the benefits of both haplobiontic life cycles | Combines the costs of both haplobiontic life cycles |

and haploid generations (diplobiontic life cycle) (for context, see Fig. 11).

## Conclusions and implications

Debates about what constitutes the basic unit of selection continue today and can be traced back to disagreements between Charles Darwin and Alfred Russel Wallace. Darwin (1859, 1872) espoused the individual organism as the unit upon which selection acted, whereas Wallace (1889) assumed that groups of individuals were the unit of selection (Ruse 2013). The authors of the synthetic theory of biological evolution sided with Darwin's view (Mayr 1982; Kutschera and Niklas 2004). Indeed, it is fair to say that the majority of today's evolutionary biologists share Darwin's perspective of what constitutes the individual. However, there are those who advocate multilevel selection theory and consider a wider spectrum of units of selection ranging from the individual cell to the level of an entire species.

Under any circumstances, it should be apparent that there are many instances where the distinction between the "individual" and the "group" becomes problematic, as for example in the case of bacterial biofilms (Fig. 12), mycelial fungi, corals, ascidians and root graphing poplar trees. Our central argument, which is presented here in the context of discussing Weismann's theory of heredity, is that different definitions are required for what constitutes an "individual" even among lineages that have evolved complex multicellularity. Indeed, even a brief review of the diversity of life histories coupled with the standard model for the transformation series of body plans leading up to multicellularity (Fig. 10) reveals that the majority of multicellular organisms fails to comply with Weismann's Doctrine and that the unit of selection can (and often does) constitute an individual cell or a cell lineage. Much like Charles Darwin, August Weismann made great contributions to biology in general and to evolutionary biology in particular. However, both men also failed in their attempts to understand the full diversity of life on Earth and the mechanism of inheritance at the sub-cellular level.

There is no criticism implied by stating this all too obvious fact. We draw attention to this short-coming only to reiterate that an export-of-fitness phase attended or followed by a germ-soma specialisation is requisite for conceiving of the organism as the "individual" in a strict Darwinian–

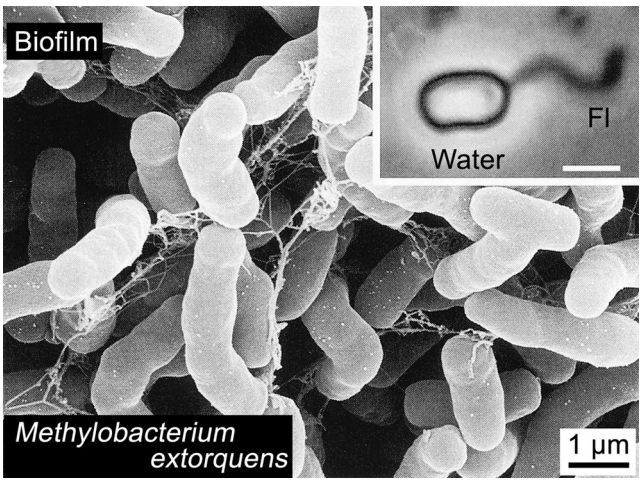


**Fig. 12** Prokaryotic microbes, such as epiphytic bacteria (*Methylobacterium* sp.) can exist, when transferred into liquid medium, as single organisms (with flagellum; *inset*). Alternatively, they live on the surface of leaves, as multicellular biofilms (without flagella; the cells are attached via extracellular polysaccharides). The unit of selection can be either the single organism (cell) or a collective of microbes (colony). Under real-world conditions, biofilms represent the dominant way of life of most bacteria, which form large collectives of interconnected cells (adapted from Schauer and Kutschera 2008)

Weismannian sense. This "one genome-in-one-body concept" of the individual is severely restricted to a comparatively small portion of all the organisms that ever inhabited our planet or that exist today (Five Kingdoms of Life). In this context, we want to stress that more than 50 % of the protoplasmic biomass on Earth consists of prokaryotic cells (bacteria, archaea and cyanobacteria) (Kutschera and Niklas 2004; Kutschera 2011, 2013). It is well known that these prokaryotic microbes, for instance the plant-associated methylobacteria (Fig. 12), usually exist as super-cellular biofilms, and only rarely in the flagellated, free-swimming (planktonic) form (Schauer and Kutschera 2008; Doerges and Kutschera 2014). Obviously, in these prokaryotic microorganisms, the "unit of selection" usually corresponds to the static biofilm, and not to the mobile, individual bacterial cells.

Finally, it should be noted that, when the pioneers of evolutionary biology, Darwin, Wallace and Weismann, published their most important works, species representative of late-divergent animal and plant lineages, such as mice, men and flowering plants were the central model organisms in the emerging sciences devoted to phylogenetic analyses (Darwin 1859, 1872; Wallace 1889; Weismann 1913). This classical "DWW-view" of "the organism" has also played an overly important role in discussions about the evolution of individuality and multicellularity. Whereas the sequestration of germ track cell lines early in an organism's ontogeny can render a congeries of cells an "individual", cellular specialisation and differentiation may play the same role. We therefore argue that cooperation among cells became an evolutionarily stable strategy that permitted "many genomes in one body", provided that one or more mechanisms evolved to counteract the incentive to cheat in the game of achieving reproductive success. This principle not only applies to eukaryotic, but likewise to prokaryotic organisms, which represent the "hidden" majority of living beings on planet Earth (Fig. 12).

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