

# Maximal Indirect Development, Set-Aside Cells, and Levels of Selection

NEIL W. BLACKSTONE<sup>1\*</sup> AND AARON M. ELLISON<sup>2</sup>

<sup>1</sup>*Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115*

<sup>2</sup>*Department of Biological Sciences, Mount Holyoke College, South Hadley, Massachusetts 01075*

**ABSTRACT** The evolution of metazoan development as described by Davidson et al. (1995. *Science* 270:1319–1325) is readily interpretable in terms of levels-of-selection conflicts, for instance, as recently modeled by Michod (1999. *Darwinian Dynamics*, Princeton, NJ: Princeton University Press). Davidson et al. propose certain features of early bilaterians including small size, a small and fixed number of cell divisions during and subsequent to cleavage, and specification of cell fates prior to cell movement. These features suggest constraints on certain parameters of Michod's model, specifically  $t$  (the time available for cell division) and  $b$  (the benefit to cells of not cooperating in terms of their rate of replication). Such constraints clearly enhance between-cell cooperation and allow multicellularity to more easily evolve and be maintained. Nevertheless, these constraints are completely abrogated by the phenomenon of "set-aside cells," that is, undifferentiated cells that retain indefinite division potential. Levels-of-selection theory predicts that the evolution of these set-aside cells must be accompanied by features which alleviate cell-cell competition, and indeed the results of Ransick et al. (1996. *Proc Natl Acad Sci USA* 93:6759–6763) support this prediction: the evolution of "set-aside cells" in metazoans was accompanied by the evolution of the sequestration of the germ line. *J. Exp. Zool. (Mol. Dev. Evol.)* 288:99–104, 2000. © 2000 Wiley-Liss, Inc.

Davidson et al. ('95) propose a hypothesis for the evolution of metazoan development with broad relevance to this new field. Davidson et al. suggest that a particular developmental pattern found in some modern marine invertebrates ("maximal indirect development") provides clues concerning the development of early bilaterian metazoans. Transcending this primitive developmental pattern may have been a major breakthrough in metazoan evolution, allowing the evolution of macroscopic body plans without coloniality. Further, the developmental events that allow metazoans to escape the limitations suggested by maximal indirect development ("set-aside cells") correlate with the determination of the germ line in modern metazoans, and these two mechanisms may have evolved in tandem (Ransick et al., '96).

This hypothesis and subsequent elaborations (Peterson et al., '97; Cameron et al., '98) have drawn widespread comment and criticism (e.g., Conway Morris, '98a,b; Davidson and Ruvkun, '99; Knoll and Carroll, '99; Wolpert, '99). Much of the discussion of this hypothesis focuses on whether the features of modern metazoans that constitute maximal indirect "Type 1" development are primi-

tive or derived. In this pattern of development, the embryo produces a larva that bears no morphological resemblance to the adult, and in modern taxa this larva is usually planktotrophic. Since set-aside cells and maximal indirect development allow larval functions to be conserved while the adult rudiment develops (e.g., Fig. 2 of Peterson et al., '97), these features might be especially advantageous in a biphasic life cycle. Nevertheless, the debate over which metazoan life cycle is primitive or derived is an old one and is unlikely to be clearly resolved (e.g., Brusca and Brusca, '90). Even with robust phylogenies, reconstructing ancestral character states from extant taxa is, at best, problematic (e.g., Cunningham et al., '98). Moreover, the character states of basal metazoans (e.g., sponges and cnidarians) are ambiguous in this regard. Basal metazoans have multipotent stem cells, but they do not have set-aside cells as

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\*Correspondence to: Neil W. Blackstone, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.  
E-mail: neilb@niu.edu

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characterized by Davidson et al. Despite the absence of such set-aside cells, basal metazoans may have planktotrophic larvae (Brusca and Brusca, '90; Gilbert and Raunio, '97). The association between maximal indirect development, set-aside cells, and planktotrophy in modern bilaterians may thus be a derived feature.

Could maximal indirect development and set-aside cells have evolved in some context other than planktotrophy? It is perhaps surprising that no attempt has been made to explicate these features in terms of the levels-of-selection conflicts attendant to the evolution and maintenance of multicellularity in animals. As with all multicellular organisms, development of metazoans must provide mechanisms to hold in check defecting cells, which selfishly favor their own replication rate over that of the multicellular group (Buss, '87, '99; Maynard Smith and Szathmáry, '95; Grosberg and Strathmann, '98; Michod, '99). These mechanisms of conflict mediation were essential for multicellularity to evolve, and they remain essential for multicellularity to be maintained (e.g., consider the lethal effects of mammalian cancers). The germ line is prominent among these mechanisms for conflict mediation. Nevertheless, the germ line is likely a derived feature in metazoans, as it is generally absent from basal metazoans and colonial protists (Buss, '87). The stem lineage of bilaterians, or independent bilaterian lineages, may plausibly have derived the germ line. Other mechanisms of conflict mediation likely also evolved in metazoans, particularly early in their history before the evolution of the germ line. In this context, we consider the hypothesis of Davidson et al. ('95), and subsequent elaborations (Peterson et al., '97; Cameron et al., '98). In particular, the consequences of maximal indirect development and set-aside cells for levels-of-selection conflicts can be seen most clearly by considering explicit population genetic models for the evolution of multicellularity (Michod, '96, '97a,b, '99; Michod and Roze, '97, 2000).

#### HYPOTHESIS OF DAVIDSON ET AL.

Davidson et al. ('95) focus on a pattern of metazoan development that they term maximal indirect development. Modern metazoans displaying this pattern generally exhibit a fixed number of divisions during cleavage, giving rise to a small larva from which the adult develops as a noticeably distinct imaginal rudiment. For instance, the early embryos of the sea urchin show a pattern of embryogenesis in which cleavage proceeds for a

set number of divisions usually in the range of  $10 \pm 2$ . By the end of cleavage, all the blastomeres are specified and each cell lineage gives rise to certain cell type(s). Cell lineage determination derives largely from the relative positions of successive cleavage planes, and the structures formed by these lineages are generally invariant within a species. Thus (Davidson et al., '95: p 1321):

Specification of the founder cells involves both conditional and autonomous mechanisms. . . . These mechanisms define the identity, positions, and differentiated fates of the progeny of all the founder cells in situ, before the embryonic cells acquire any capability for cell motility.

The larvae thus formed are small, perhaps a few hundred or thousand cells, as illustrated by the sea urchin, *Strongylocentrotus purpuratus* (Davidson et al., '95: p 1320):

At the end of the larval stage, there are about 150,000 cells, or about 100 times more than at the end of embryogenesis . . . , but more than 90% of these are included in the adult rudiment. After embryogenesis, the cells of the larva itself replicate only two or three more times, on average, during the whole of postembryonic larval life.

Davidson et al. suggest that this sort of maximal indirect development is a widespread, general, and basic mode of development in modern bilaterian animals. While common and widespread is clearly not equivalent to plesiomorphic, maximal indirect development is certainly characteristic of echinoderms, which are basal bilaterians (Aguinaldo and Lake, '98). This pattern of development is also found in a number of lophotrochozoan taxa. Based on these data, Davidson et al. hypothesize that the entire ontogeny of early bilaterians was equivalent to the embryogenesis of these modern taxa. In other words, the bilaterian stem lineage may have matured at a stage comparable to the larva of those modern species undergoing maximal indirect development. In this sense, "Type 1" embryogenesis in modern taxa is a vestige remaining from this stem lineage. It is illuminating to note several features of this hypothetical early bilaterian and the relationship of these features to levels-of-selection conflicts. A multicellular animal with its entire ontogeny equivalent to the maximal indirect larval devel-

opment would have (1) cell fate determined prior to the movement of any cells, and subsequently (2) a very limited number of divisions of each cell lineage. In terms of conflicts between cell lineages, this mode of development has clear implications for minimizing both the chance for the origin of a variant cell (by minimizing the number of cell divisions) and the potential gains of such a cell if it did indeed arise (by determining cell fate prior to the possibility of cell movement).

The developmental features that Davidson et al. attribute to the bilaterian stem lineage may thus have evolved precisely to resolve the levels-of-selection conflicts inherent in the evolution and maintenance of multicellularity, and the success of early bilaterians may have depended on these features. For instance, these early bilaterians were likely competing with contemporaneous multicellular organisms which likely included multicellular protists, perhaps superficially similar to the modern *Volvox* or *Proterospongia*, and clonal or colonial basal metazoans. In all these forms, organismal fitness likely was (and is) diminished by constant competition between cell lineages (Buss, '87; Blackstone, 2000; Michod and Roze, 2000). Bilaterians were likely derived from colonial metazoans (e.g., Dewel, 2000). Nonetheless, the derived features of the development of the bilaterian stem lineage (described by Davidson et al.) may have transcended the levels-of-selection conflicts inherent in colonial life cycles.

In transcending these costs of coloniality, however, stem bilaterians also had to forgo the benefits of large size. According to Davidson et al., significant changes in this archetypal developmental pattern were necessary to produce the body plans of modern macroscopic bilaterians. Specifically, these macroscopic bilaterians had to evolve a system in which a transient pattern of transcription factor expression is imposed on a field of undifferentiated cells which then replicate repeatedly, with the numerous progeny of these cells ultimately developing the adult structures. The key evolutionary innovation was a group of undifferentiated "set-aside cells" on which novel patterns of gene expression could subsequently evolve to act. As described by Davidson et al. ('95: p 1323):

We conceive the most important evolutionary novelty to have been the developmental use of yet undifferentiated set-aside cells, which retain indefinite division potential, as a substrate for the morphogenesis of large

structures. . . . Among the genetic regulatory changes required to produce set-aside cells are the disconnection of the cell division controls that are a prominent feature of Type 1 embryos. . . .

Thus in order to produce the body plans of macroscopic bilaterians, qualitatively different developmental regulatory mechanisms had to evolve. These new mechanisms in fact abrogated the very features of early bilaterian development which putatively limited competition between cell lineages.

### MICHOD'S MODEL

The transition in evolution from single cells to multicellular organisms, and the subsequent maintenance of multicellularity, has attracted considerable attention (Buss, '87, '99; Maynard Smith and Szathmáry, '95; Bonner, '98; Grosberg and Strathmann, '98). To provide a quantitative framework for generating and evaluating hypotheses for this transition, Michod ('96, '97a,b, '99) and Michod and Roze ('97, 2000) explicitly model the conditions related to the emergence and maintenance of the multicellular organism as a distinct level of selection. This multilevel selection model includes genetic mutation and selection within and between organisms under different reproductive modes and parameter values. Michod shows that selection and mutation during development generate significant levels of within-organism variation and lead to significant variation in organismal fitness over a wide range of parameter values. Multicellularity flourishes when the fitness covariance at the level of the organism overcomes within-organism change toward "defecting" cells (Michod, '97a,b). Michod ('96) and Michod and Roze ('97) investigate the conditions under which a modifier locus, which alters the parameters of selection and variation within the multicellular organisms, could evolve. Such a modifier locus could control germ line formation as discussed below.

In terms of these models, the bilaterian stem lineage of Davidson et al. represents a way of minimizing parameters subsumed by  $t$ , the time available for development, and  $b$ , the benefit or replicatory advantage of a "defecting" or "selfish" somatic cell. Most of the cell divisions in this hypothetical stem bilaterian occur during the cleavage of the zygote where maternal controls of cell division can override any advantage of a selfish variant (Buss, '87). Thus, in such a metazoan not only is the time of maternally-independent devel-

opment extremely short, consisting of only two or three further cell divisions, but the replicatory advantage of a variant cell is constrained by the specification of cell fate before cell movement is possible. The latter hinders, for instance, the capacity of a variant cell lineage to become systemic. A small  $t$  and  $b$  allow between-cell cooperation and multicellularity to more easily evolve and flourish (e.g., Fig. 5 of Michod, '97a). The parameters subsumed by  $t$  and  $b$  may have posed very real constraints in the history of multicellular life. Early bilaterians may have evolved such a pattern of development precisely for these reasons (e.g., contrast this pattern to the development of *Volvox*, *Proterospongia*, slime molds, or basal metazoans; Buss, '82, '87, '99; Bonner, '98; Grosberg and Strathmann, '98; Blackstone, 2000; Michod and Roze, 2000).

Nevertheless, the features of this hypothetical bilaterian stem lineage which ensure the successful resolution of levels-of-selection conflicts are completely abrogated by the developmental breakthroughs discussed by Davidson et al. A population of "set-aside cells" with both unlimited replicatory potential and full mobility would greatly increase  $t$  and  $b$ . For multicellularity to persist, an enhanced  $\beta$ , the advantage to an adult organism of cooperation among its cells, would be required. Large size would provide such an increased advantage (Michod, '97a). Nevertheless, undifferentiated set-aside cells would remain a threat to successful multicellularity (Michod, '97a: p 638): "Whatever its advantage, large size means more cells, and more cells means more opportunity for mutation during development."

Consider the germ line in this context. As pointed out by Michod ('97a: p 612): "One hypothesized function of the germ line. . . is to uncouple the organism's fitness from the fitness of the cells comprising it. . . ." Specifically, the germ line derives from a modifier locus, which alters the parameters of selection and variation within the multicellular organism by sequestering gamete-producing cells from somatic cells early in development (Michod, '96; Michod and Roze, '97). Gamete-producing cells thus have a different developmental history than somatic cells, dividing fewer times and perhaps exhibiting a different rate of mutation. A germ line also has a fitness cost to the organism in that gamete-producing cells do not contribute to cooperative somatic functions. Models incorporating these considerations show that it is easier for a germ line to evolve when  $t$  and  $b$  are large (Fig. 1 of Michod and Roze,

'97). Such parameter values are precisely the consequences of set-aside cells.

The findings of Ransick et al. ('96) are thus perhaps unsurprising: in modern metazoans with maximal indirect development (p 6761) "the definitive germ line segregates in the context of adult body plan formation" and (p 6762) "in these animals the germ line arises *de novo* only after the embryo and larva have completed their own development, from the same pool of unspecified cells that give rise to the rest of the adult body." These results imply that germ line determination evolved in the stem lineage of bilaterians in concert with the developmental innovations which ultimately permitted large size, although it is also possible that these features were derived in tandem by independent bilaterian lineages. While it remains an open question as to which was the more important innovation, the correlated evolution of set-aside cells and the germ line suggests a prominent role for levels-of-selection conflicts in the history of bilaterian (and metazoan) development.

## CONCLUSIONS

It is perhaps an understatement to say that difficulties confront attempts to infer evolutionary events that occurred during the early evolution of multicellular animals. The physical environment at this time may have been markedly different from the present (Knoll and Carroll, '99; Kerr, 2000). Transitional forms likely became extinct long ago (Maynard Smith and Szathmáry, '95; Bonner, '98), and experiments on extant, derived forms must be interpreted with caution (Conway Morris, '95). While an improving fossil record (Conway Morris, '98b; Knoll and Carroll, '99) offers increasing insight, this major evolutionary transition and related events still remain largely opaque.

Thus while extrapolating from patterns in the development of extant organisms to evolutionary events that occurred hundreds (or thousands) of millions of years ago is not automatically valid (Conway Morris, '95), developing an understanding of these events will necessarily require the use of all available data. In particular, the cellular natural histories of multicellular organisms such as those elucidated by Davidson et al. ('95) and Ransick et al. ('96) can and should play an important role in testing models of the evolution of the multicellular individual (Michod, '96, '97a,b, '99; Michod and Roze, '97, 2000). It is quite satisfying that these very distinct fields of inquiry lead to at least a qualitative confluence. For instance,

Michod's model predicts that the abrogation of the features of "Type 1" embryogenesis by "set-aside cells" should be accompanied by other mechanisms of conflict mediation in bilaterians, and Ransick et al.'s results suggest that this was accomplished via the evolution of the germ line.

This synthesis also allows a reconciliation of the Davidson et al. hypothesis with the widespread criticism of the notion that planktotrophy is primitive in bilaterian metazoans (e.g., Conway Morris, '98a,b; Wolpert, '99). The stem lineage of bilaterians may have evolved a tightly constrained development (represented in modern metazoans by the vestigial "Type 1" embryogenesis) to escape the costs of the levels-of-selection conflicts that hindered other early multicellular eukaryotes. Multicellular protists and basal metazoans certainly exhibit none of these "Type 1" constraints (Buss, '87), and the mechanisms of conflict mediation in these multicellular organisms remains an area of active investigation (Blackstone, 2000; Michod and Roze, 2000). While bilaterians were derived from colonial metazoans (e.g., Dewel, 2000), the key to the early success of this lineage may have been precisely those features that now constitute "Type 1" embryogenesis. Nevertheless, in transcending the costs of coloniality in terms of levels-of-selection conflicts, stem bilaterians also had to forgo the benefits that coloniality provided in terms of large size. Set-aside cells and a sequestered germ line may have subsequently evolved in bilaterians to escape the constraints of small size while still holding in check the costs of levels-of-selection conflicts. Maximal indirect development and set-aside cells may thus have evolved independently of planktotrophic larvae. By this view, maximal indirect development and set-aside cells are plesiomorphic features of the bilaterian stem lineage, or possibly independent bilaterian lineages, while planktotrophic larvae are derived. The plesiomorphic features of maximal indirect development and set-aside cells may be retained in those modern bilaterians for which they have some other particular advantage, i.e., those taxa that subsequently derived biphasic life cycles and planktotrophic larvae.

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