The Evolutionary Origin of an Altruistic Gene

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Although the conditions favoring altruism are being increasingly understood, the evolutionary origins of the genetic basis for this behavior remain elusive. Here, we show that reproductive altruism (i.e., a sterile soma) in the multicellular green alga, *Volvox carteri*, evolved via the co-option of a life-history gene whose expression in the unicellular ancestor was conditioned on an environmental cue (as an adaptive strategy to enhance survival at an immediate cost to reproduction) through shifting its expression from a temporal (environmentally induced) into a spatial (developmental) context. The gene belongs to a diverged and structurally heterogeneous multigene family sharing a SAND-like domain (a DNA-binding module involved in gene transcription regulation). To our knowledge, this is the first example of a social gene specifically associated with reproductive altruism, whose origin can be traced back to a solitary ancestor. These findings complement recent proposals that the differentiation of sterile castes in social insects involved the co-option of regulatory networks that control sequential shifts between phases in the life cycle of solitary insects.

Altruism—the capacity to perform acts costly to self but beneficial to others—is central to the evolution of various biological phenomena from social behavior to evolutionary transitions in individuality (Maynard Smith and Szathmáry 1997). Although the selective conditions favoring altruism are being increasingly understood, the evolutionary origins of the genetic basis for this behavior remain elusive.

Reproductive altruism is the most extreme form of altruism and is best typified by the existence of castes in eusocial insects and sterile somatic cells in multicellular organisms (e.g., Buss 1987; Maynard Smith and Szathmáry 1997; Queller 2000; Frank 2003). The most important assumption underlying the evolution of reproductive sterility concerns conditionality; that is, the expression of sterility genes must be conditioned on some cue—such that those who express sterility benefit others who do not express it but do transmit it (Queller 2000). It has been suggested that in multicellular groups which develop from a single cell, a sterile soma could evolve if a mutation that altruistically removes its bearers from the germ line is expressed conditionally, in a developmental context (Queller 2000).

The evolution of soma was instrumental to the increase in complexity in multicellular lineages—as by altruistically removing themselves from the reproductive line, somatic cells could specialize in various survival-related functions. We have previously proposed that the evolution of germ-soma separation required a change in the expression of vegetative and reproductive functions from a temporal into a spatial context (Nedelcu and Michod 2004). Here, we argue that the evolution of soma involved the co-option of life-history genes whose expression in the unicellular ancestors was conditioned on environmental cues (as an adaptive strategy to enhance survival at an immediate cost to reproduction) through shifting their expression from a temporal (environmentally induced) into a spatial (developmental) context.

To address this hypothesis, we used Volvocales—a group of closely related photosynthetic flagellated green algae comprising both unicellular species (e.g., *Chlamydomonas reinhardtii*) as well as multicellular forms with up to 10 000 cells (e.g., some species of *Volvox*) (Kirk 1998). In the latter, the increase in cell number is paralleled by an increase in the number of cells that do not reproduce (fig. 1*A*). Why would these cells give up the right to reproduce? The conditions favoring the evolution of an altruistic soma in these algae are generally understood in the context of 1) developmental constraints (i.e., due to the presence of a coherent glycoproteic cell wall, flagellar basal bodies cannot move laterally and act as centrioles while still remaining attached to the flagella—thus, flagellar motility is impeded during cell division, especially in larger colonies whose reproduction requires a high number of cell divisions; Koufopanou 1994), 2) ecological pressures (e.g., flagellar activity is required to maintain themselves in the water column at an optimum position relative to sun light intensity; Kirk 1998, p 70), and 3) advantages associated with a division of labor (e.g., motility while reproducing and increased growth rate due to a source-sink effect and local flagellar mixing; Kirk 1998, p 61–4; Solari, Kessler, and Michod 2006; Solari et al. 2006).

What is the genetic basis for reproductive altruism in these algae, and could its evolutionary origin be traced back to their unicellular ancestor? The most studied multicellular volvocalean species, *Volvox carteri*, consists of approximately 2000 permanently biflagellated somatic cells and up to 16 nonflagellated reproductive cells. Terminal differentiation of somatic cells in *V. carteri* involves the expression of *regA*—a master regulatory gene that encodes a transcriptional repressor (Kirk et al. 1999) thought to suppress several nuclear genes coding for chloroplast proteins (Meissner et al. 1999). Consequently, the cell growth (dependent on photosynthesis) and division (dependent on cell growth) of somatic cells are suppressed. Because they cannot divide, they do not participate directly in the offspring but contribute to the survival and reproduction of the colony (Kirk 1998, p 62–4; Solari, Kessler, and Michod 2006; Solari et al. 2006)—in the same way that sterile workers do in a social insect colony. In other words, the somatic cells express an altruistic behavior, and *regA* (whose expression is necessary and sufficient for this behavior; Kirk et al. 1999) is an altruistic gene. Consistent with being an altruistic gene (Queller 2000), *regA* is expressed conditionally, in a developmental context. Which cells do not express *regA* and

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differentiate into germ cells is determined early during embryonic development through a series of asymmetric cell divisions (restricted to the anterior hemisphere of the embryo). The asymmetric divisions ensure that some cells (i.e., the germ line precursors) remain above the threshold cell size associated with the expression of *regA* (Kirk 1995).

As with all forms of cooperation, this altruistic behavior is also susceptible to defection and selfish mutants; indeed, mutations in *regA* result in the somatic cells regaining reproductive abilities,which in turn results in them losing their flagellar capabilities (e.g., Kirk et al. 1987). As motility is very important for these algae (discussed earlier), the survival and reproduction of *V. carteri* individuals in which such mutant somatic cells occur is negatively affected (Solari, Kessler, and Michod 2006); this is supported by the absence of Reg mutants as established populations in nature, although they occur spontaneously at a rather high rate (Kurn et al. 1978).

To address the possibility that *regA* in *V. carteri* evolved from a gene already present in its unicellular ancestor, we searched the *C. reinhardtii* genome v3.0 database at the Joint Genome Institute (http://genome.jgi-psf.org/Chlre3/Chlre3.home.html) for sequences with similarity to *regA*. Interestingly, such sequences (of unknown function) were found on several (at least 5) scaffolds (*Crsc1*, *Crsc8*, *Crsc13*, *Crsc49*, *Crsc59*), suggesting the presence of a multigene family (fig. 1*B*). Our finding multiple *regA*-like sequences is consistent with the recent report of a *regA*-like sequence in *V. carteri* (*rlsA*; GenBank accession number: AF106962; fig. 1*B*); furthermore, we identified several additional *regA*-like sequences in the available *V. carteri* genome data (accessible through the *C. reinhardtii* genome database) (not shown),which supports the idea of a *regA* gene family. However, *regA* is known as the only locus that can mutate to yield Reg mutants (Kirk et al. 1999).

Noteworthy, with the exception of an approximately 80–amino acid region (fig. 1*B* and *D*), the *V. carteri* and *C. reinhardtii* predicted RegA-like sequences are quite diverged. InterProScan (http://www.ebi.ac.uk/InterProScan/) and SMART (http://smart.embl-heidelberg.de/) identified the 80–amino acid conserved region as being similar to the SAND domain (IPR000770; PF0134)—which is found in a number of nuclear proteins, many of which function in chromatin-dependent or DNA-specific transcriptional control (http://www.ebi.ac.uk/interpro/IEntry?ac=IPR000770). SAND-containing proteins have a modular structure, and the SAND domain is believed to mediate the DNA-binding activity of these proteins. Although initially thought to be restricted to animal phyla, proteins containing a SAND domain have been recently reported in land plants; one such protein, ULTRAPETALA1, acts as a key negative regulator of cell accumulation in *Arabidopsis* shoot and floral meristems (Carles et al. 2005).

In addition to the SAND-like domain, the RegA-like sequences all share an abundance of A, G, Q, E, P organized in "multiplets"(e.g., fig. 1*E*); an abundance of A, Q, and/or P is a unifying characteristic of active transcriptional repressors (including the *V. carteri* RegA)—which otherwise have little discernible structural similarities (Kirk et al. 1999). Lastly, no transmembrane segments, or mitochondrial/chloroplast target peptides, but potential nuclear localization signals have been predicted by SignalP (http://www.cbs.dtu.dk/services/SignalP/), TargetP (http://www.cbs.dtu.dk/services/TargetP/), and PSORT (http://www.psort.org/).

The *V. carteri* and *C. reinhardtii regA*-like sequences are also quite different in gene structure, both in terms of the number/size of exons and location of introns as well as the relative position of the conserved SAND-like region (fig. 1*B*). The number of introns varies from none in *Crsc49* to 5 in *Crsc8* and, except for the introns present in the conserved region (which are inserted at homologous positions in at least 2 sequences), have an apparent random differential distribution (fig. 1*B*). Several families of transcription factors (e.g., the nuclear receptor and the GATA family) are also known to belong to multigene families whose members (or subfamilies) differ dramatically in their exon–intron structure and number and location of intronic sequences (Reyes et al. 2004; Zhang et al. 2004).

Due to limited sequence information and lack of expression data (to confirm the exon–intron boundaries as well as the functionality of these sequences), it is not possible at this time to address specific questions concerned with the mechanisms associated with the evolution of this gene family (which is also outside the scope of this report). Nevertheless, several suggestions can be made. 1) The fact that the similarity among *regA*-like paralogs is restricted to the SAND-like domain is consistent with partial duplication events. 2) As some of the exons in the *C. reinhardtii regA*-like sequences and all exons in the *V. carteri regA* are symmetrical (fig. 1*B*), exon shuffling can also be invoked in some cases (Patthy 1987). 3) However, as no hits were found when the unique exons were blasted against the available genome sequences of *C. reinhardtii* and *V. carteri*, recruitment of neighborhood sequences is likely to have also been involved in the evolution of at least some of the current exons. In this context, it is noteworthy that a recent analysis of the pathways leading to the formation of novel genes via duplication in the nematode *Caenorhabditis elegans* found that partial duplications in conjunction with recruitment of neighborhood sequences exceeded chimeric duplications and argued for a major role that these processes play in creating structurally novel genes from extant genomic material (which can lead to immediate acquisition of novel adaptive functions) (Katju and Lynch 2006). 4) The *regA* locus exhibits stage-specific hypermutability in response to agents that cause error-prone recombination/repair (Kirk et al. 1987), and Reg mutants can also be easily induced by the transposition of *Jordan*—a *V. carteri* mobile element stimulated by several types of stress (Miller et al. 1993; Kirk et al. 1999). Transposable elements are known to promote gene rearrangements (inversions, translocations, insertions, and duplications), and several families of retrotransposon and transposon sequences have been described in *V. carteri* (e.g., Miller et al. 1993; Duncan et al. 2002). Thus, it is possible that some of the rearrangements associated with the evolution of *regA*-like sequences in the lineage leading to *V. carteri* have been transposon mediated.

To determine which *C. reinhardtii regA*-like sequence is most closely related to the *V. carteri regA*, we performed phylogenetic analyses using the SAND-like domain. Both Bayesian (fig. 1*C*) as well as maximum likelihood and

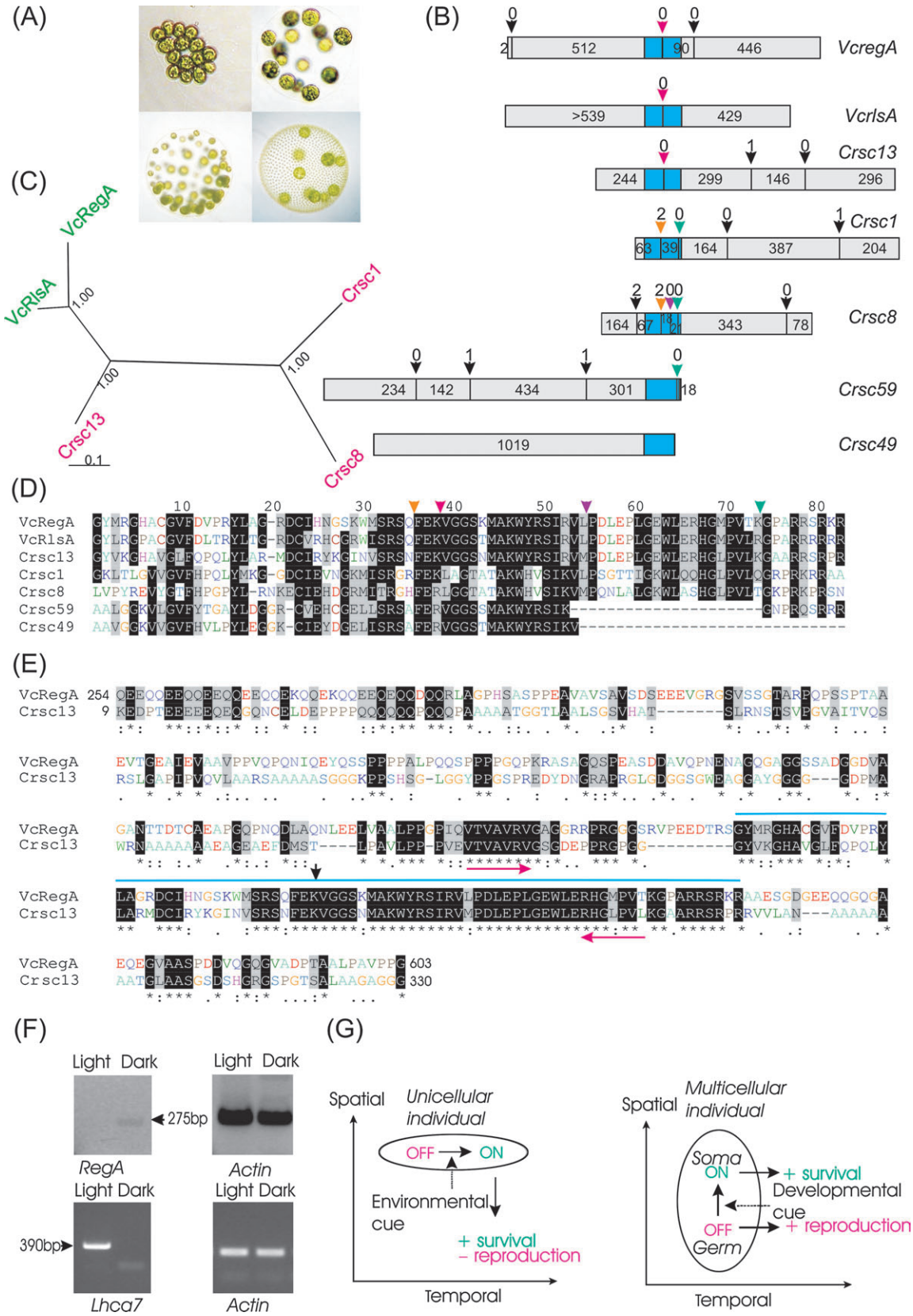

FIG. 1.—(*A*) Multicellular volvocalean algae without (upper panels: *Gonium pectorale* and *Eudorina elegans*) and with (lower panels: *Pleodorina californica* and *Volvox carteri*) somatic cells (smaller cells are somatic biflagellated cells; larger cells are nonmotile reproductive cells). (*B*) Gene structure of 2 *V. carteri* (*VcregA* and *VcrlsA*; GenBank AF106962) and 5 *Chlamydomonas reinhardtii* (*Crsc1*, *Crsc8*, *Crsc13*, *Crsc49*, and *Crsc59*; Protein IDs in the *Chlamydomonas* v3.0 database: 146989, 141155, 145132, 152832, and 153500, respectively) *regA*-like sequences (genes are aligned vertically);

parsimony (not shown) analyses indicate Crsc13 as being the *C. reinhardtii* RegA-like sequence most closely related to the *V. carteri* RegA/RlsA clade. This is also consistent with these 3 sequences sharing a splice junction in their SAND-like domain (fig. 1*B*); note that the other 2 introns present in the *V. carteri regA* translated region, and missing in *Crsc13*, contain cell-type regulatory elements (Stark et al. 2001). A close relationship between Crsc13 and *V. carteri* RegA is further indicated by the fact that amino acid similarity extends outside the SAND-like domain (fig. 1*E*).

Notably, the *V. carteri* RegA and RlsA sequences are more closely related to each other than either is to any of the *C. reinhardtii RegA*-like sequences (fig. 1*C*) , suggesting that *V. carteri regA* and *rlsA* are the result of a duplication event that took place in the lineage leading to *V. carteri*. This is supported by the fact that while no 2 of the *C. reinhardtii regA*-like sequences are located on the same scaffold, the *V. carteri regA* and *rlsA* sequences are adjacent (separated by a ca. 1.3-kb intergenic region; GenBank accession number: AF106962).

The presence of *regA*-like sequences in *C. reinhardtii* is puzzling at first; why would a unicellular individual suppress its own reproduction? Interestingly, despite several expressed sequence tag sequencing projects using RNA extracted from *C. reinhardtii* cultures grown in various conditions (http://www.chlamy.org/libraries.html; http://www.kazusa.or.jp/en/plant/chlamy/EST/), no evidence of any of these *regA*-like sequences being expressed was found. Are these sequences mere pseudogenes? If not, when might these genes be expressed? As *V. carteri regA*'s expression is associated with the suppression of chloroplast biogenesis (Meissner et al. 1999), we predicted that—if functional, the *Crsc13 regA*-like sequence (indicated by our analyses as being most closely related to *V. carteri regA*) must be expressed when photosynthetic activities and chloroplast biogenesis have to be down regulated. To simulate such conditions, we grew *C. reinhardtii* in the dark (note that *C. reinhardtii*, but not *V. carteri*, is mixotrophic and can grow on acetate) and investigated the expression of both the *regA*-like sequence and a nuclear gene coding for a chloroplast protein, *lhca7*, thought to be a RegA target in *V. carteri* somatic cells (Meissner et al. 1999). As predicted, the *C. reinhardtii Crsc13 regA*-like is specifically induced in the dark, and its expression coincides with the down regulation of *lhca7* (fig. 1*F*).

What is the functional and adaptive significance of these findings? In both plants and green algae, photosynthetic activities and chloroplast protein composition are adjusted in response to various environmental changes, as an adaptive mechanism (photosynthetic acclimation) that can enhance survival (Grossman 2000; Walters 2005)—especially in variable environments such as those in which volvocalean algae live (Kirk 1998). Dynamic changes in chloroplast composition are thought to ensure unnecessary investment in particular sets of proteins, thus releasing resources for use in other cellular processes (Grossman 2000; Walters 2005). Although here we manipulated light, genes encoding chloroplast components can be regulated by factors other than light (including nutrient stress). Recently, phosphate deprivation was shown to trigger complete suppression of 2 genes coding for chloroplast proteins in plants (Jain et al. 2005), one of which—*psbO* is a potential RegA target in *V. carteri* (Meissner et al. 1999) (noteworthy, phosphate is the limiting nutrient in the environments in which volvocalean algae thrive; Kirk 1998, p 56–60).

Thus, our finding that the *C. reinhardtii Crsc13* is induced in an environment in which chloroplast biogenesis is restricted suggests that this *regA*-like gene in *V. carteri*'s unicellular ancestor was likely induced under environmental conditions when the temporary down regulation of chloroplasts was beneficial in terms of survival. As cessation of cell division is another hallmark of acclimation (Grossman 2000), *regA*'s expression was likely costly in terms of immediate reproduction. Such a life-history gene (i.e., a gene that benefits survival while detracting from immediate reproduction—with the effect over the life cycle being beneficial) can become altruistic in the context of a multicellular group, if the beneficial effect of this gene is also beneficial to the group and if the cell as a group member expends more effort on this beneficial effect than would be optimal for its own survival and reproduction. As shown in figure 1*G*, this can be realized by shifting this gene's expression pattern from a temporal context (within the same cell) into a spatial context (between soma and germ). In this way, a life-history gene that trades-off survival and reproduction in a unicellular individual can become an altruistic gene in a multicellular group and create the conditions for individuality at the higher level to emerge.

To our knowledge, this is the first example of a social gene specifically associated with reproductive altruism, whose origin can be traced back to a solitary ancestor. The finding that the evolution of germ-soma separation in *V. carteri* involved the co-option of a life-history gene present in its unicellular ancestor complements and provides

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numbers indicate the predicted exon size (in amino acids). The SAND domain is shown in blue, and the introns are shown as arrows (same color arrows denote shared insertion sites; 0, 1, 2 indicate intron phases). (*C*) Bayesian analysis using the region shown in figure 1*D* (numbers represent posterior probability distributions of trees; Huelsenbeck and Ronquist 2001). (*D*) The predicted amino acid sequences corresponding to the conserved region shown in blue in figure 1*B*. (*E*) Partial alignment between the *V. carteri* RegA and the *C. reinhardtii* Crsc13 RegA-like predicted amino acid sequences (overlining indicates the conserved region in 1*B*; vertical arrow denotes the location of the shared intron; horizontal arrows indicate the location of the *regA*-like primers used in 1*F*). (*F*) Expression patterns of the *Crsc13 regA*-like and *lhca7* (protein ID 78552 in the *Chlamydomonas* v3.0 database); induction of *regA*-like (upper panels) and down regulation of *lhca7* (lower panels) in cultures grown for 3 days in the dark. *C. reinhardtii* CC-2454 was obtained from the *Chlamydomonas* Center (http://www.chlamy.org/). Cultures were grown in TRIS-acetate-phosphate medium (Gorman and Levine 1965), under a 12:12 h light:dark cycle (the "light" treatment) or wrapped in aluminum foil (the "dark" treatment). RNA extraction and reverse transcriptase–polymerase chain reaction were carried out as described in Nedelcu et al. (2004). Primers were designed across introns to ensure that amplification products are from RNA only; actin was used as a control. (*G*) Schematic representation of the change in expression pattern from a temporal context (environmentally induced) into a spatial context (developmentally induced) of a life-history gene in a unicellular individual as it becomes an altruistic gene in a multicellular individual.

support to recent proposals that cast differentiation and social evolution in insects involved the remodeling of regulatory circuits present in solitary ancestors (e.g., Robinson et al. 2005)—such as those controlling the bivoltine life cycle (an adaptation to seasonal environments) in the solitary ancestors of the eusocial wasp *Polistes* (Hunt and Amdam 2005). Generally, sociality is thought to have evolved through modifying molecular mechanisms associated with the perception and processing of environmental stimuli by solitary organisms (Robinson et al. 2005). In this context, our findings that the evolution of an altruistic soma involved the co-option of a regulatory pathway conditioned on environmental cues argue that the 2 biological phenomena, although involving vastly different lineages, are based on a common selective framework. With the rise of sociogenomics (Robinson et al. 2005), this recognition should help identify general principles underlying the evolution of sociality and evolutionary transitions in individuality at the molecular level.

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