Allard's argument versus Baker's contention for the adaptive significance of selfing in a hermaphroditic fish

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Fertilization assurance (Baker's contention) and multilocus coadaptation (Allard's argument) are two distinct hypotheses for the adaptive significance of self-fertilization in hermaphroditic taxa, and both scenarios have been invoked to rationalize isogenicity via incest in various plants and invertebrate animals with predominant selfing. Here we contrast Allard's argument and Baker's contention as applied to the world's only known vertebrate that routinely self-fertilizes. We pay special attention to frequencies of locally most common multilocus genotypes in Floridian populations of the Mangrove Rivulus (*Kryptolebias marmoratus*). Isogenicity patterns in this fish appear inconsistent with Allard's argument, thus leaving Baker's contention as the more plausible scenario (a result also supported by natural history information for this species). These results contrast with the isogenicity patterns and conclusions previously drawn from several self-fertilizing plants and invertebrate animal species. Thus, the adaptive significance of selfing apparently varies across hermaphroditic taxa.

Baker's rule | coadapted genes | androdioecy | reproductive assuance

The mating system of many hermaphroditic plants and invertebrate animals includes at least occasional self-fertilization (1–4). Why selfing is so common poses an evolutionary enigma because self-fertilization is an extreme form of incest that often reduces genetic fitness via inbreeding depression (5). Why then do so many dual-sex organisms routinely self-fertilize? One standard explanation is that the transmission advantage of selfing compared with outcrossing is sufficient to outweigh inbreeding depression and thus enable selfing to invade a population of outcrossers (6).

Within that context, two major classes of adaptive benefit have been argued to help explain selfing's evolutionary maintenance. The first hypothesis follows logically from the severe restriction on genetic recombination that self-fertilization promotes (7). When selfing predominates in a sexual population for even a few generations, heterozygosity rapidly decays (homozygosity increases) such that relatively little intraindividual genetic variation soon remains available for shuffling into novel multilocus allelic suites. In the extreme, reproduction within a highly inbred lineage in effect becomes "clonal" as highly homozygous individuals self-fertilize in successive generations and thereby produce essentially isogenic (genetically identical) offspring. Although meiosis and syngamy continue to operate in a selfing lineage, these cellular processes become ineffective in generating recombinant genotypes; instead, multilocus isogenotypes favored by natural selection tend to be preserved and proliferate (Fig. 1).

In the early 1970s, Robert Allard and colleagues published seminal articles empirically documenting how selfing's restriction on genetic recombination can act in conjunction with natural selection to favor the spread of coadapted multilocus suites of alleles well molded to local ecological conditions (7, 8–11). These studies involved plant populations with predominant selfing and showed that particular multilocus genotypes routinely reached substantial frequencies in populations with high self-fertilization rates. Furthermore, as summarized by Allard et al. (12), "these multiallelic configurations are distributed ecogeographically in patchwork patterns that are precise overlays of environmental

heterogeneity" and thus "natural selection was the predominant integrating force in shaping the specific genetic structure of different local populations as well as the adaptive landscape." With regard to purported evolutionary benefits of self-fertilization as a tactical component of a mixed-mating strategy, for shorthand we will refer to this genomic-coadaptation scenario as Allard's argument for the adaptive significance of selfing.

Soon thereafter, Robert Selander and colleagues published an analogous series of studies documenting multilocus coadaptation in local populations of terrestrial hermaphroditic snails with high selfing rates (13–16). Again, specific multilocus suites of alleles appeared to be coadapted to particular ecological conditions and thereby often were driven to moderate or high frequencies in populations of these invertebrate animals.

Long before the works of Allard and Selander, however, Herbert Baker (17, 18) had proposed a very different hypothesis for the adaptive significance of self-fertilization, especially in the context of establishment after long distance dispersal (e.g., weeds, island colonization). According to Baker (19), fitness payoffs from selfing derive primarily from fertilization assurance [also known as "reproductive assurance" (20–22)] during island colonization or after long distance dispersal into distant habitats where conspecifics might not be available for outbreeding. (This advantage also had been appreciated by Darwin.) Unlike obligate outcrossers that lack the capacity to reproduce without a mate, each self-compatible individual is reproductively self-sufficient. Baker interpreted fertilization assurance to be the key advantage of selfing, an argument that gained empirical support from documented associations—across plant species (23, 24), and particularly for island species (25) and also among invertebrate animal taxa (26)—between proclivity to self-fertilize and colonization potential. Baker argued that self-fertilization is advantageous in any hermaphroditic species in which opportunities for outcrossing are constrained for any reason, such as low population density (e.g., in newly colonized habitats), unequal sex ratio, or any other ecological or genetic basis for a paucity of suitable mates. We henceforth refer to fertilization assurance as Baker's contention for the adaptive significance of selfing. Allard's argument and Baker's contention are not mutually exclusive (i.e., both could apply to a given species), but they do convey different sentiments about fitness dividends from self-fertilization. Under Allard's argument, selfing's restraint on genetic recombination is the key evolutionary factor, whereas Baker's scenario emphasizes inherent benefits of selfing that derive from assured fertilization.

Table 1 summarizes several other implications of Baker's contention versus Allard's argument. For example, the former implies that selection might act to favor even a single gene that promotes self-fertilization, whereas the latter invokes coadaptation at the genomic level. Thus, although fertilization assurance (Baker's contention) offers immediate fitness payoffs to
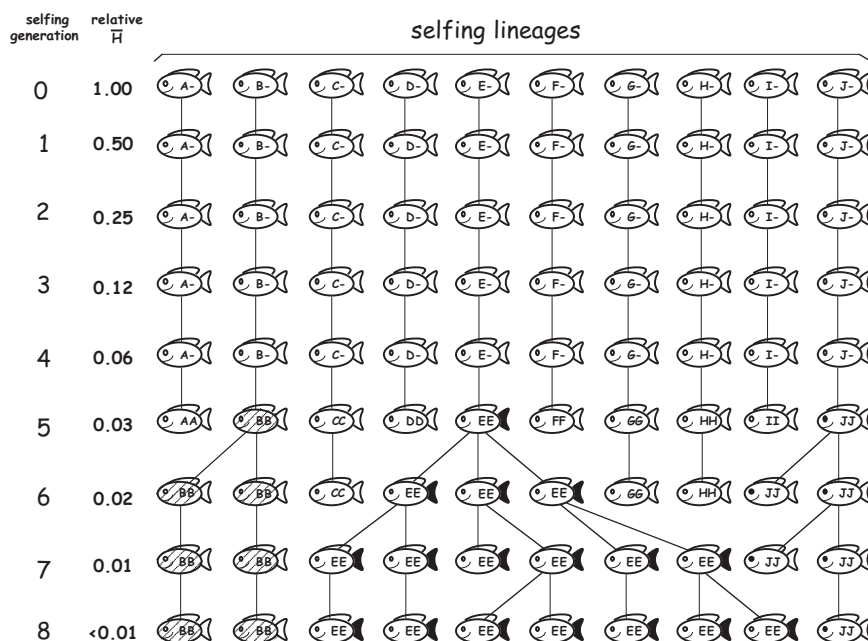
Fig. 1. Expected declines in population $\bar{H}$ and shifts in the frequencies of isogenic genotypes across eight generations of selfing, starting from an outbred deme in generation 0. Multilocus genotypes that are effectively isogenic begin to emerge by generation 5; some of these may soon proliferate under the influence of natural selection (and/or genetic drift). For example, isogenic lineage EE (black-tailed fish) represents only 10% of the population in generation 5 but increases to 70% of the population by generation 8. Similarly, isogenotype BB (striped body) increases to 20% of the population by generation 8. The demise of any isogenic lineage (such as the termination of FF in generation 5 or the disappearance of CC in generation 6) can be interpreted to register either reproductive failure or an outcross event by the focal individual(s).

any self-fertile individual in each generation, especially when mating opportunities are limited, Allard's argument entails deferred fitness benefits because several generations of selfing and natural selection must transpire before a formerly outcrossed lineage might evolve multilocus coadaptation (either inside the genome or in relation to external ecological niches). Furthermore, further outcrossing effectively disintegrates (via genetic recombination) any coadapted multilocus genotype that selection and selfing may have collaborated to sculpt across successive generations of selfing.

With regard to the incidence of isogenicity in selfing species, Allard's argument implies that the frequencies of various multilocus genotypes should register "interclonal" selection, whereas Baker's contention implies that any such isogenotypes should reflect differential proliferation via dispersal and colonization. With regard to the spatial arrangement of isogenotypes, Allard's

argument implies that particular multilocus genotypes should reach moderate to high frequencies in habitats where they are locally well adapted. Empirically, in Allard's self-fertilizing plants and in Selander's self-fertilizing snails, different multilocus genotypes consistently characterized local populations occupying xeric versus mesic environments. By contrast, Baker's scenario necessitates no epistasis and no particular association between multilocus genotype and habitat. Indeed, Baker's contention invokes no coadaptation, either across multiple loci or between particular isogenotypes and specific environmental conditions.

Because various authors have used biological data from plants and invertebrates to argue in favor of either Allard's argument or Baker's contention, a fresh perspective from other selfing taxa might be helpful. Here we examine multilocus genotypes in the world's only known vertebrate clade in which hermaphrodites routinely self-fertilize. Observed distributions of isogenotypes in

Table 1. Evolutionary features of Allard's argument versus Baker's contention for the adaptive significance of selfing

Feature	Allard's argument	Baker's contention
Nature of selection	For genes coadapted to one another in a given habitat	Fertilization assurance
Epistasis implied?	Yes	No
Level of selection	Multilocus genotype	Any gene that promotes selfing
Timing of selective benefits	Deferred for several generations postoutcrossing	Immediate in each selfing generation
Recipient of genetic benefits*	The inbred lineage	Each selfing individual
Isogenicity implied?	Yes	Not necessarily
Frequency of isogenotypes	Often locally high	Seldom locally high except after dispersal
Arrangement of isogenotypes	Often associated with particular habitats	Often disjunct or perhaps patchily distributed
Selfing ↔ fitness	Fitness benefits are a consequence of selfing	Fitness benefits favor the evolution of selfing

*One fundamental distinction between Baker's contention and Allard's argument is that the former emphasizes selfing's immediate fitness payoffs to the individual, whereas the latter invokes only deferred fitness dividends that might accrue from self-fertilization extended across multiple generations. In other words, fertilization assurance (under Baker's contention) offers immediate fitness payouts to any self-fertile individual in each and every generation, especially when mating opportunities are limited for any reason. By contrast, multilocus genetic coadaptation (under Allard's argument) offers deferred and perhaps tenuous fitness benefits to the initiating individual because any outcrossing within an inbred lineage immediately undoes what selection and selfing may have combined to accomplish across at least several successive generations of strict selfing within an inbred lineage.

EVOLUTION

Kryptolebias marmoratus (Cyprinodontiformes; *Kmar*) as well as the natural history of this piscine clade appear to be more consistent with Baker's than with Allard's scenario.

Background

Kryptolebias Lineage. The Mangrove Rivulus belongs to a small killifish clade that includes several closely related taxa with proclivities for hermaphroditism and in some cases self-fertilization (27, 28). Selfing by *Kmar* was discovered in the early 1960s (29), and in nature it yields highly homozygous lines composed of individuals so genetically uniform as to be, in effect, clonally identical to one another (30). Thus, *Kmar* became a model for studying the evolutionary ramifications of self-fertilization and clonality in a vertebrate species (reviewed in ref. 31). The following are some of genetic discoveries made across the years about these unique fish: (*i*) selfing lineages in *Kmar* do exhibit genetic variation, albeit at reduced levels compared with most outcrossing species (32–37); (*ii*) males as well as hermaphrodites exist in some populations (38, 39), thus making *Kmar* an androdioecious species (40); (*iii*) these males sometimes mediate outcross events (41) such that *Kmar* has a mixed-mating system (40, 42, 43) with predominant selfing; and (*iv*) these and other features of the reproductive system have been mapped phylogenetically and phylogeographically across members of the *Kmar* clade to yield inferences about evolutionary histories of the self-fertilization syndrome (27, 44, 45).

Here we summarize microsatellite and other biological data that may have some bearing on Allard's versus Baker's scenarios for the proximate adaptive significance of selfing in *Kmar*. Specifically, we address the degree to which particular multilocus isogenotypes are present and how they are geographically arranged in highly selfed populations of this species. One prediction from Allard's argument is that local environmental conditions should favor coadapted isogenic lineages, which therefore should be common at particular locales. Baker's scenario, by contrast, implies that isogenotypes might be rare and perhaps geographically disjoined.

Isogenicity. *Distribution of isogenicity in theory.* Fig. 2 outlines a theoretical framework for our discussions of isogenicity in selfing species. Imagine a baseline population in which each hermaph-

roditic individual begins to self-fertilize in generation (G) = 0. In each successive generation of selfing in this increasingly inbred population, heterozygosity (H) declines by 50%, eventually reaching <1% of its initial value by about $G = 7$ (Fig. 2, *Left*). Thus, only after $G \sim 7$ of selfing can reproduction within an inbred lineage begin to yield offspring whose multilocus genotypes are essentially identical to one another and to that of the selfing parent. At the outset of such isogenicity, the expected population frequency of each such multilocus genotype should be $\sim 1/N$, assuming selective neutrality. Thereafter, the expected frequency of the locally most common multilocus genotype (LMCMLG) in a local population can begin to change in response to evolutionary forces such as genetic drift or natural selection. If we initially assume that each local population is closed to outside gene flow and is large and stable in size, then the effects of genetic drift can be neglected and the evolutionary dynamics become governed by selective forces operating in conjunction with the breeding system. If we further assume that selfing continues at a high rate and that natural selection favors any particular multilocus genotype (MLG) over all others, then the frequency of the LMCMLG should increase in sigmoidal fashion and eventually approach fixation as shown by the trajectories in the shaded portion of Fig. 2, *Right*. The temporal duration of any such evolutionary transition from low to high frequency of an LMCMLG is a function of the intensity of favorable selection acting on each LMCMLG. However, an important point is that the frequency of the LMCMLG in any population is expected to be nontrivially high (>0.10) across a broad range of parameter values (selection coefficients and numbers of generations elapsed since the onset of isogenicity at $G \sim 7$) (Fig. 2). In other words, Allard's argument implies that particular multilocus genotypes favored by natural selection should often be present in moderate to high frequencies in local populations of any species with high rates of self-fertilization.

Distribution of isogenicity in practice. The theory outlined in this article implies that Allard's argument predicts moderate to high frequencies for selectively favored MLGs in local populations of predominant selfers. Do such expectations match empirical data? For Allard's self-fertilizing plants and Selander's self-fertilizing snails, the answer is a resounding "yes": in almost every

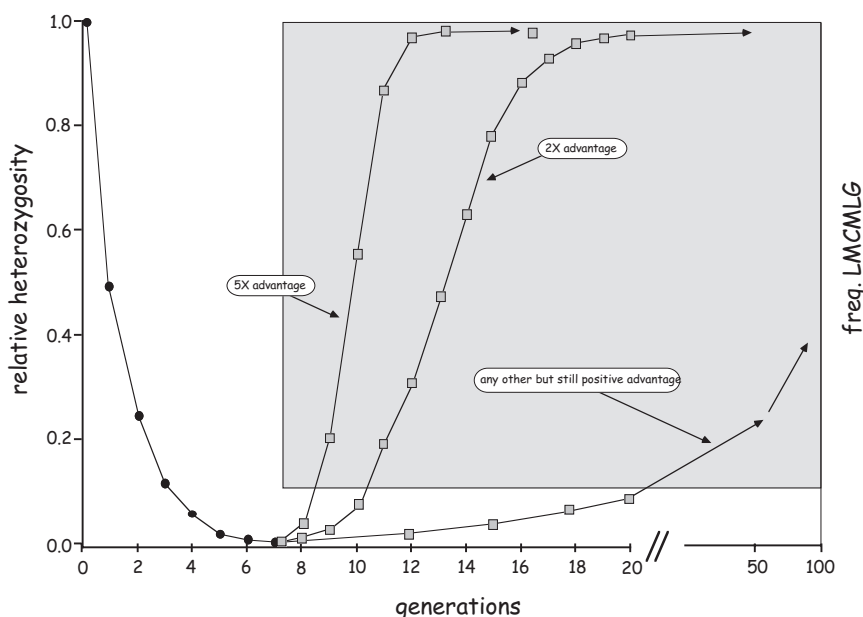**Fig. 2.** Theoretical evolutionary dynamics of heterozygosity (the black-dot curve, *Left*) and LMCMLG frequencies (the three curves, *Right*) in a population of self-fertilizing hermaphrodites. The shaded box indicates the broad range of positive adaptive values and generation times under which expected frequencies of the LMCMLG are expected to be rather high (i.e., >0.10) under Allard's scenario.

Avise and Tatarenkov

population, one or another MLG was quite common or even fixed locally (Fig. 3). In *Kmar*, by contrast, LMCMLGs almost never reached moderate or high frequencies (Fig. 3). Indeed, only seldom did MLGs occur in a local *Kmar* population other than as singletons (Figs. 3 and 4). Among the total of more than 150 different MLGs observed in the current study, in no case has natural selection apparently driven a given MLG to high frequency in any local *Kmar* population surveyed.

Evolutionary implications of contrasting isogenicity patterns. One possible explanation for the paucity of locally common isogenotypes in *Kmar* is that outcrossing events in these fish are far more common than previously suspected. This seems unlikely however because selfing rates have been estimated both from direct genetic assays of progeny in the laboratory (40) and indirect genetic evidence from natural populations (27, 43). Furthermore, males (who presumably mediate the outcross events) are documented to be rare in Florida in field surveys that now span nearly half a century (30, 45).

A second possibility for the paucity of common MLGs in *Kmar* is that populations in Florida were founded too recently for isogenicity to have been achieved and for favorable selection on particular MLGs to have taken hold. This seems unlikely however because isogenicity via selfing requires only about half-

Fig. 3. Frequencies of the LMCMLG in populations of *Kryptolebias marmoratus* [*n* = 12 Floridian populations (refs. 37 and 45), *Arabidopsis thaliana* [*n* = 39 (ref. 46)], *Avena barbata* [*n* = 50 (ref. 12)], and *Rumina decollata* [*n* = 4 [ref. 16)]. Only samples with 10 or more assayed individuals are included.
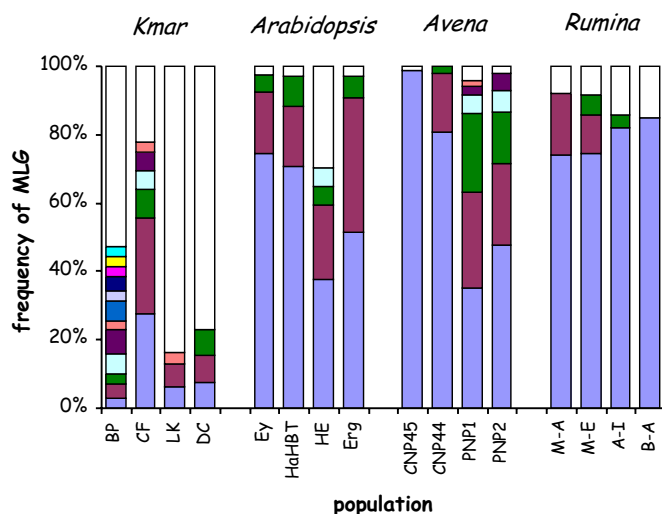
Fig. 4. Proportionate representations of different multilocus genotypes in four representative local populations each of *Kmar*, *Arabidopsis*, *Avena*, and *Rumina*. White regions at the top of each bar register pooled singleton genotypes, which were common in *Kmar* but generally much less so in assayed populations of the other three self-fertilizing species.

dozen generations to arise (Fig. 2), self-fertilizing *Kmar* populations are known to have inhabited South Florida for at least 30 y, and *Kmar* has a short generation time [i.e., three to four generations per year (47, 48)]. A third possibility for the low frequency of LMCMLGs in *Kmar* is genetic drift in small populations. Although we cannot exclude this possibility, under Allard's scenario, natural selection should interact with and perhaps even override genetic drift even in small populations.

A fourth possibility for the low frequency of LMCMLGs in *Kmar* is that microsatellite markers are selectively neutral and hence not directly subject to selection. However, this explanation misses the broader point that selfing restricts recombination throughout the genome, including at neutral loci, thereby generating strong gametic-phase disequilibrium and potentially making particular MLGs quite visible to natural selection. Nevertheless, it remains possible that favorable gene combinations ("coadapted gene complexes") might well emerge very early in a selfing population—perhaps well before more variable loci approach isogenicity.

A fifth and related possibility for the paucity of common MLGs in *Kmar* is that genetic assays for *Kmar* were more refined than those for Allard's plants or Selander's snails. Allard's studies on plants and Selander's studies on snails involved

allozymes, which typically show far less polymorphism than do the microsatellite loci we used for *Kmar*. To address whether this technical factor alone might account for the disparity in isogenicity patterns between *Kmar* and other self-fertilizing taxa, we added to our analysis a genetic survey of another self-fertilizing plant: the wild mustard *Arabidopsis thaliana*. Bomblies et al. (46) used 436 SNPs to assess multilocus population genetic structure in 77 local populations of this species (which also displays <5% outcrossing). Despite the high discriminatory power of these SNPs, frequencies of LMCMLGs in *A. thaliana* often proved to be moderate to high, much as for Allard's plants and Selander's snails (Fig. 3). Thus the striking contrast in isogenicity patterns between *Kmar* and these other self-fertilizers seems unlikely to be due solely to different powers of resolution by the molecular markers used. This implies that some biological (rather than technical) factor(s) must have shaped the distinct isogenicity outcomes in *Kmar* versus the self-fertilizing plants and invertebrates included in this survey.

Allard's Argument Versus Baker's Contention for *Kmar*? We found no locally common multilocus isogenotypes in *K. marmoratus*. As demonstrated in this article, this isogenicity pattern departs dramatically from those in several other self-fertilizing species previously assayed, and this disparity cannot readily be ascribed to differences in outcrossing rates or genetic assays that were applied to these fishes, plants, and invertebrates, all of which have mixed-mating systems with high selfing rates. The paucity of fixed or even common multilocus genotypes in local populations of *Kmar* seems inconsistent with Allard's argument for the adaptive significance of self-fertilization in this species. Therefore, partly by default, genetic patterns in *Kmar* appear to be more compatible with Baker's contention for the adaptive significance for selfing, an interpretation arguably reinforced by several natural history facets of *Kmar* biology (Table 2). Although definitive evidence for (or against) Allard's scenario ultimately must come from direct appraisals of the relative genetic fitness of multilocus genotypes in particular habitats, at present the balance of genetic and other biological evidence seems to favor fertilization assurance (Baker's contention) as offering the better explanation for any adaptive benefits that have attended the evolutionary maintenance of self-fertilization in the *Kmar* clade.

This is not to imply that Allard's argument carries little force for the self-fertilizing plants and invertebrate animals in which genomic coadaptation was strongly implicated by several lines of theoretical and empirical evidence in addition to patterns of isogenicity per se. Overall, our current comparisons indicate that different adaptive scenarios for selfing probably apply across diverse taxa with similar rates of self-fertilization. Thus, whether Allard's or Baker's contention best accounts for the adaptive

Table 2. Natural history evidence supporting Baker's contention for adaptive significance of *Kmar* clade selfing

i) Factors that imply limited mating options (and thus place fitness premiums on selfing:
  a) Kmar appears to be quite uncommon at most locales within its range.*
  b) Hermaphrodites greatly outnumber gonochoristic males at most sites.
  c) Individuals often tend to be relatively solitary.*
  d) Individuals tend to be highly belligerent toward conspecifics.
ii) Factors that imply a strong colonization potential:
  a) The clade has a broad geographic distribution [southern Florida, most islands of the Caribbean (including the Bahamas), and the Atlantic coast from Yucatan to southeast Brazil].
  b) *Kmar* inhabits coastal environs that are prone to strong storms and ocean currents.
  c) Individuals have been taken in large numbers inside rotting mangrove logs that obviously could be transported as flotsam.
  d) Adults can survive out of water for several weeks (when packed in wet material such as moist leaves or plant debris).
  e) Adults can move short distances on land (wet mangrove forest floors) by flipping.
  f) The species probably practices facultative egg stranding in which fertilized eggs may be stranded for a time before hatching (although the extent of embryonic diapause under field conditions remains unknown).

*However, *Kmar* natural history is poorly known, and some studies suggest that the species may occur in substantial aggregations and be social at particular times or sites (49, 50).

benefits of clonality via incest ultimately may have to be decided for each taxon case by case. Furthermore, the current study makes it clear that information on the frequencies of isogenotypes will be necessary to decide between Allard's and Baker's scenarios in any taxon. Other model genetic organisms that regularly self-fertilize, such as some nematodes in the genus *Caenorhabditis*, should be amenable for critical experimentation on this topic.

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EVOLUTION