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Evolutionary perspectives on clonal reproduction in vertebrate animals

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A synopsis is provided of different expressions of whole-animal vertebrate clonality (asexual organismal-level reproduction), both in the laboratory and in nature. For vertebrate taxa, such clonal phenomena include the following: human-mediated cloning via artificial nuclear transfer; intergenerational clonality in nature via parthenogenesis and gynogenesis; intergenerational hemiclonality via hybridogenesis and kleptogenesis; intragenerational clonality via polyembryony; and what in effect qualifies as clonal replication via self-fertilization and intense inbreeding by simultaneous hermaphrodites. Each of these clonal or quasi-clonal mechanisms is described, and its evolutionary genetic ramifications are addressed. By affording an atypical vantage on standard vertebrate reproduction, clonality offers fresh perspectives on the evolutionary and ecological significance of recombination-derived genetic variety.

cloning | asexuality | unisexuality | parthenogenesis | polyembryony

As these symposium proceedings will attest, clonal reproduction is widely recognized to be an important component of the reproductive repertoires in many pathogenic microbes, plants, and invertebrate animals (1). Less widely appreciated is the fact that various expressions of clonal phenomena also occur in the vertebrate realm, both under human and natural auspices. Here I briefly summarize research on the many varied manifestations of clonal and quasi-clonal reproduction (as defined in Box 1) by animals with backbones. The findings are of biological interest in their own right and are also relevant to our broader understanding of the ecological and evolutionary significance of alternative reproductive modalities. For more comprehensive treatments of clonal phenomena in vertebrate animals, readers are directed to refs. 2–4.

Vertebrate Clonality Under Human Auspices

Beginning in the early 1950s, embryologists working with frogs developed laboratory techniques by which they could microsurgically transfer the intact nucleus from a somatic cell of a tadpole or adult frog into an artificially enucleated frog egg (5, 6). Such nuclear transplantation (NT) experiments yielded egg cells that began to divide and multiply mitotically, eventuating in a new generation of offspring each of which was a clonal replica of its nuclear-donor parent. Evidently, under some circumstances, the nuclear genome from a differentiated donor cell could begin to act again like a totipotent stem cell to direct full embryonic development. These experiments surprised nearly everyone, because previously it was supposed that the genome of a liver cell (for example) had undergone tissue-specific changes in gene regulation that would have caused it to lose its pluripotency (capacity to give rise to many different cell types in a growing individual). In 2012, John Gurdon was awarded a Nobel Prize in Physiology or Medicine for his pioneering role in stem-cell research and NT cloning.

In 1997, Wilmut et al. (7) extended NT cloning to another vertebrate species when they produced the famous lamb Dolly, the world's first NT-generated mammalian clone. Since that time, researchers have NT cloned a wide variety of domestic and other backboned animals, ranging from laboratory mice (8) and aquarium fishes (9), to house pets (10, 11) and farm animals (12, 13), and even to some to endangered species (14, 15). Although NT cloning of humans (*Homo sapiens*) proved to be technically somewhat more difficult, the ethically fraught task of cloning human cells was finally accomplished in 2013 (16).

The line between artificial and natural cloning sometimes blurs because nature in effect also deploys NT cloning occasionally, as for example under parthenogenesis when an egg cell receives an unreduced nuclear genome and begins to proliferate mitotically into a daughter organism that is genetically identical to her mother. The demarcation between artificial and natural cloning can blur even further when we take into account the fact that, for several vertebrate taxa, researchers occasionally have induced parthenogenesis, gynogenesis, hybridogenesis, or polyembryony by various experimental procedures, such as forced hybridization, exposure to UV radiation or exotic chemicals, microsurgical embryonic splitting, or intense inbreeding in the laboratory (3).

Unisexual Vertebrates and Intergenerational Clonality in Nature

About 100 "species" of vertebrate animal are known to consist solely of females who produce daughters that are genetically identical (barring de novo mutations) to one another and to their mother. Such an all-female taxon may not qualify as a biological species in the usual sense of that term, because it is not held together by the anastomotic bonds of mating that otherwise apply to standard bisexual species. Thus, the phrase "unisexual biotype" (rather than species) is typically applied to male-absent taxa that reproduce without benefit of sex. These unisexual biotypes reproduce via parthenogenesis (virgin birth), gynogenesis, or hybridogenesis, all of which therefore constitute intergenerational expressions of clonality or hemiclonality. Black and white drawings of many of these species can be found in ref. 3.

Constitutive Parthenogenesis. All of the known vertebrate practitioners of constitutive (consistent) parthenogenesis reside in the taxonomic order Squamata (lizards, snakes, and allies). Examples include several rock lizards (especially in the genus *Darevskia*) of the family Lacertidae (17), various geckos in the family Gekkonidae (18), whiptail lizards (especially in the genus *Aspidoscelis*) of the family Teiidae (19), skinks in the family Scincidae (20), and a blind snake in the family Typhlopidae (21). For each such unisexual biotype, reproduction transpires when a female produces chromosomally unreduced eggs that then proliferate mitotically to form daughter individuals that display the same

genetic constitution as the mother. No males or paternal gametes (sperm) are required to complete this strictly clonal operation.

Facultative Parthenogenesis. In recent years, several instances of sporadic parthenogenesis (tychoparthenogenesis) have come to light in species that otherwise reproduce sexually. For example, in the Burmese snake, *Python molurus* (22), and in the Bonnethead shark, *Sphyrna tiburo* (23), captive specimens without known access to males occasionally have given virgin birth to progeny that have proved on molecular examination to be genetically identical to their one and only female parent. I strongly suspect that many additional instances of tychoparthenogenesis (either meiotic or ameiotic) have gone unnoticed in nature simply because suitable molecular markers have not been systematically deployed to address possible clonal identities in large numbers of individuals from most natural vertebrate populations.

Gynogenesis. This reproductive mode is quite like constitutive parthenogenesis except that a sperm cell is required to activate or stimulate mitotic divisions in each unreduced egg such that embryonic development ensues to produce a daughter that again is clonally identical to her gynogenetic mother. [This phenomenon is quite similar to what is observed in some types of pseudogamous apomictic plants, in which pollen is required to stimulate asexual seed production sometimes from related species (24).] In vertebrates, the usual source of such activating sperm for the unisexual (all-female) biotypes that engage in gynogenesis is also a related bisexual species that otherwise reproduces intraspecifically by standard sexual means. These heterospecific males are said to be "sexually parasitized" by the gynogenetic females because they make no actual genetic contributions to their interspecific "daughters." In short, gynogenesis is quite like the reproductive process of parthenogenesis but with an added dash of nonrecombinational sex.

Most of the known vertebrate practitioners of gynogenesis are various unisexual fishes and amphibians. Approximately 50 named species of sperm-dependent unisexual vertebrates have been described. Gynogenetic taxa can be found for example in live-bearing fishes of the family Poeciliidae (25), silverside fishes in the family Atherinidae (26), several minnows in the family Cyprinidae (27), loach fishes in the family Cobitidae (28), some cyprinodontid killifishes (29), and mole salamanders in the family Ambystomatidae (30).

Hybridogenesis. This reproductive mode generally resembles gynogenesis except for the following: (*i*) the egg cell that each female produces is meiotically reduced (typically haploid) and carries only the maternal set of chromosomes; (*ii*) a sperm cell (from a sexually parasitized heterospecific mate) actually fertilizes that egg and contributes paternal-origin chromosomes to it, thus transiently reestablishing the diploid (or higher) ploidy level in the progeny; and (*iii*) these paternally derived chromosomes are then jettisoned via an abnormal meiosis during oogenesis in each daughter. Thus, only the maternal suite of nuclear chromosomes is clonally transmitted across successive generations in this "hemiclonal" or quasi-sexual reproductive system. This outcome also means that a sexually parasitized male can be a genetic father but not a genetic grandfather of the resulting offspring. Otherwise, hybridogenesis resembles gynogenesis in the sense that both systems typically entail unisexual (all-female) biotypes that are sperm dependent and enlist participation by sexually parasitized males from a related taxon. Hybridogenesis characterizes some live-bearing fishes in the family Poeciliidae (31), some cyprinid minnows (32), some mole salamanders in the family Ambystomatidae (33), and various European water frogs in the family Ranidae (34).

Kleptogenesis. A few unisexual vertebrate biotypes in nature do not fit easily into the categories parthenogenesis, gynogenesis, or hybridogenesis. Members of the salamander genus *Ambystoma* provide prime examples (35). So too do some ranid frogs (36). For some of these populations, genetic markers reveal that the unisexual biotypes sometimes incorporate bits of nuclear DNA from locally sympatric sexual species into their diploid or polyploid nuclei. In other words, such otherwise clonal taxa appear to capture or "steal" some nuclear DNA from local congeners. Some of these hybridogenetic systems have also been described as genetically "leaky." The mechanistic routes to such genomic thievery in these or other such taxa are not entirely clear, but the mere fact that kleptogenetic (37) biotypes exist highlights the notion that some unisexual vertebrates are not strictly clonal or hemiclonal but instead probably gain some genetic benefits from occasional recombinational sex. The fact must be borne in mind when assessing the apparent evolutionary durations of particular forms of clonal or quasi-clonal reproduction.

Evolutionary and Genetic Origins. Without exception, all unisexual vertebrate biotypes arose via interspecific hybridization. The species that hybridized to produce the parthenogens, gynogens, hybridogens, or kleptogens invariably were congeneric bisexuals, but often they were not sister taxa (closest genetic relatives within the sexual clade). This situation means that all unisexual biotypes are genetically highly variable (heterozygous at most or all nuclear loci that distinguish their respective parental taxa), notwithstanding the fact that they have a paucity or absence of sexual recombination once formed. Abnormal functional interactions between the amalgamated heterospecific genomes probably account for why clonal or hemiclonal taxa have such peculiar gametogenetic mechanisms; the genomes of the hybridizing species that yield clonal derivatives must have been divergent enough to disrupt the cellular mechanics of gametogenesis in progeny yet not so divergent as to seriously compromise hybrid viability or fertility. For nearly all unisexual vertebrate biotypes, researchers have used diagnostic molecular markers from nuclear and mitochondrial genomes (sometimes in conjunction with field knowledge and other evidence) to document the particular bisexual species and the direction(s) of hybridization (with respect to sex) that produced each clonal or hemiclonal taxon (25, 38).

Such molecular markers have also been used to reveal several details regarding the cytogenetic pathways by which various diploid and polyploid unisexual biotypes originated (39–43). For example, for any triploid parthenogen, the formational hybridization event might in theory either have preceded or followed the production of an unreduced egg by a diploid female. The former possibility was known as the spontaneous origin hypothesis (44, 45), whereas the latter was referred to as the primary hybrid hypothesis (46). These two hypotheses yield distinct and testable predictions about the particular combinations of nuclear and mitochondrial genomes from the two parental species in a polyploid unisexual lineage. When these predictions were put to empirical test in several specific instances (involving various unisexual lizards and fishes), the primary hybrid model often has been supported and the spontaneous origin model provisionally rejected (40, 41, 47).

Ecological and Evolutionary Success. Conventional wisdom might hold that unisexual vertebrates should be ecologically unsuccessful and evolutionarily short lived because they mostly lack the recombinational genetic variety that presumably is necessary for continual adaptation to changing environments (45) and/or because they lack meiotic mechanisms to repair DNA damage (48) and purge deleterious alleles that should accumulate under Muller's ratchet (49). Is this prediction borne out by the available evidence? Many unisexual vertebrates do indeed have narrow

geographical distributions. For example, most of the parthenogenetic biotypes in the lizard genus *Aspidoscelis* are confined to narrow ranges in the deserts of southwestern North America, as are *Darevskia* unisexuals in the Caucasus mountains of Eurasia. On the other hand, asexual races of *Heteronotia binoei* are distributed across much of the Australian continent, and some parthenogenetic geckos in the genera *Lepidodactylus* and *Nactus* inhabit many widespread islands in the Indo-Pacific region. For parthenogenetic unisexuals, the capacity of each individual to reproduce without a mate in effect provides a form of "fertilization insurance" that undoubtedly facilitates the colonization of new geographical areas. Furthermore, both in theory and in reality (50), some heterozygous genotypes that become clonally "frozen" (51) during the origination of a unisexual lineage may from the outset be fortuitously adaptive in a particular ecological niche. Empirically, some unisexual biotypes greatly outnumber their sexual relatives where they occur in sympatry. However, sperm-dependent unisexuals (gynogens and hybridogens) are in an especially precarious evolutionary position: to survive long term they must do well demographically, but not too well lest they ecologically outcompete their sexual relatives and thereby precipitate their own demise (by causing the extinction of their gonochoristic relatives and thereby losing necessary access to their sexually parasitized males).

In recent decades, many molecular genetic surveys have been conducted to address the evolutionary origins and ages of unisexual vertebrates. Mitochondrial (mt) DNA has been especially informative in this regard (52), because, for any unisexual biotype, the matrilineal history recorded in this maternally inherited system is in principle one and the same as the entire organismal phylogeny through which all genes (including nuclear loci) have been transmitted. This situation contrasts strikingly with the situation in sexual species, wherein a matrilineal genealogy records only a minuscule fraction of a species' total genetic legacy. In the molecular appraisals of evolutionary ages for unisexual taxa, two general genetic yardsticks have been used: the magnitude of postformational genetic variation within a monophyletic clonal lineage (the supposition being that the buildup of such genetic variation is a time-dependent evolutionary process) and the magnitude of genetic divergence between a monophyletic clonal lineage and its closest sexual relative (the supposition being that such divergence also registers the time elapsed since evolutionary origin).

In most cases, the mitochondrial surveys have revealed that a particular unisexual biotype comprises only a single and relatively small evolutionary branch embedded within the phylogeny of the sexual species that provided its female parent in the original hybridization event(s). In other words, each sexual ancestor typically has proved to be paraphyletic with respect to its unisexual derivative, thus suggesting a relatively recent origin and spread for the unisexual biotype. Indeed, in 12 of 13 such analyses (92%), mtDNA nucleotide diversities were dramatically higher in the bisexual species than in its respective unisexual derivative (52). Furthermore, for the great majority of 24 pairs of unisexual biotypes and their cognate sexual species examined for mitochondrial phylogeny, the inferred evolutionary ages of the clones or hemiclones were much less than 1 My (based on a standard molecular clock calibration for mtDNA). Some of the exceptional cases at face value implied origination dates up to several million years ago for a unisexual biotype (53), but such inferences might well be biased dramatically upward because the relevant bisexual ancestors might have gone extinct or otherwise remained unsampled in the phylogenetic appraisals. The fact also remains that no unisexual lineage of vertebrates has adaptively radiated into multiple taxonomic species or otherwise participated in the macroevolution of any broader all-female clade. Perhaps the maximum well-documented geological age reported for any extant vertebrate clonal or hemiclonal line is about 60,000 generations (54), but in evolutionary terms, this

duration is "but an evening gone" (55). In summation, molecular, as well as ecological and distributional, data suggest that unisexual clonality in vertebrate animals can best be viewed as a genetic operation that sometimes offers a highly successful tactic in the ecological short term but that almost invariably fails as a long-term evolutionary strategy. Of course, a demonstrably young evolutionary age for most extant unisexuals does not imply that the clonal phenomenon itself began only recently in evolution; clonal lineages probably have been arising and then rather quickly going extinct throughout the long evolutionary histories of squamate reptiles and fishes.

Polyembryony: A Sexual and Intragenerational Form of Clonality

Monozygotic "twinning" or polyembryony is an intra- rather than an intergenerational expression of clonality. It involves sexual reproduction and it yields siblings that are genetically identical to one another (barring de novo mutation) because their ontogenetic development traces back through mitotic divisions to a single fertilized egg. The phenomenon is relatively common in invertebrate animals (56) and plants (57). Sporadic polyembryony is taxonomically widespread in vertebrates also; diverse sexual species are occasionally known to produce monozygotic twins (or even triplets or quadruplets). In humans, ∼1% of successful pregnancies result in the birth of twins, and in about one-third of those cases, the twins are monozygotic. Such polyembryonic offspring are of special interest for the information they can provide about genetic vs. environmental impacts on phenotypic traits. However, sporadic polyembryony is of limited intrigue from an evolutionary vantage because, arguably, no selective explanation needs to be invoked when polyembryos are merely produced sporadically in an otherwise nonpolyembryonic species.

Of greater interest to evolutionary biologists are species that consistently or constitutively produce polyembryos. Clonality via polyembryony is a common or regular occurrence in more than a dozen invertebrate genera ranging from various bryozoans and cnidarians to some echinoderms, platyhelminthes, and arthropods (58). For such species, an evolutionary paradox arises (59): why would natural selection favor the regular production of clonemates within a clutch, as opposed to genetically diverse offspring? This reproductive enigma has been likened to the purchase of multiple lottery tickets with the same number, even though no reason exists to prefer one number over another (60). In polyembryony, the parents' full evolutionary wager for each litter in effect is placed on just one "Xeroxed" genotype. Furthermore, because that genotype was sexually produced and differs from those of both of the offsprings' sexual parents, at the outset, it is functionally untested and ecologically unproven (unlike the case for the intergenerational clonal genotypes perpetuated by parthenogenesis). Indeed, constitutive polyembryony seems at face value to combine some of the worst elements of sexuality and clonality.

Among vertebrate animals, constitutive (consistent) polyembryony is confined to long-nosed armadillos in the genus *Dasypus* (61). The best-studied species is the nine-banded armadillo, *D. novemcinctus*, which has a broad distribution in the Americas. Typically, a female produces a litter of four monozygotic pups, although litter sizes of two, three, or six have also been reported. Polyembryony in *Dasypus* initially was suspected from indirect field and laboratory evidence (such as the regular occurrence of same sex litters encased in a single chorionic membrane), but this clonal phenomenon recently was confirmed from direct molecular genetic appraisals (62) and skin grafting experiments (63) as well.

Why do nine-banded armadillos consistently produce polyembryonic litters as opposed to nonclonal offspring? One theoretical possibility involves kin selection. Perhaps polyembryos in an armadillo litter are highly nepotistic (e.g., by foraging together or

Box 1. Synoptistical glossary of relevant terms (elaborated in the text) that define various natural forms of clonality or hemiclonality in vertebrate animals

Clonality, of or pertaining to clones.

Clone (noun), a biological entity (e.g., gene, cell, or multicellular organism) that is genetically identical to another; alternatively, all genetically identical entities that have descended asexually from a given ancestral entity; (verb) to produce such genetically identical entities or lineages. In this paper, the focus is on biological entities at the level of multicellular vertebrate organisms.

Gynogenesis, reproduction in which a sperm cell is needed to activate cell divisions in an oocyte but the resulting offspring carry nuclear DNA only from the female parent.

Hemiclone, the portion of a genome that is transmitted intact, without recombination, in a hybridogenetic lineage.

Hybridogenesis, a quasi-sexual form of reproduction in which egg and sperm fuse to initiate embryonic development, but germ cells in the offspring later undergo an abnormal meiosis in which the resulting gametes carry no paternally derived genes.

Inbreeding, the mating of kin; multigenerational self-fertilization is an extreme expression of inbreeding.

Kleptogenesis, reproduction by gynogenesis-type or hybridogenesis-type mechanisms but with at least occasional incorporation of sperm-derived DNA into an otherwise clonal lineage.

Parthenogenesis, the development of an individual from an egg without fertilization.

Polyembryony, the production of genetically identical offspring within a clutch or litter (i.e., from a single fertilized egg).

Self-fertilization (selfing), the union of male and female gametes from the same hermaphroditic individual.

Tychoparthenogenesis, sporadic or facultative parthenogenesis.

perhaps collaborating to build dens or deter predators), in which case polyembryony might have been favored for the evolutionary advantages it confers via selection pressures operating on exceptionally close kin. However, behavioral observations in the field and laboratory have yielded no indication of nepotism among *Dasypus* littermates (64). Thus, researchers have focused on other kinds of evolutionary explanations for the polyembryony phenomenon in armadillos. Two developmental peculiarities of *Dasypus* are notable. The first is embryonic diapause or delayed implantation, in which the armadillo blastocyst—a postzygotic cell mass— undergoes a quiescent period of several months before implanting into the pregnant female's uterus. Early speculation was that embryonic diapause might directly cause polyembryony, e.g., by altering maternal or embryonic physiology in ways that somehow prompt polyembryonic divisions of the blastocyst (65). However, delayed implantation also characterizes many other mammals that are not polyembryonic—such as some seals, bears, skunks, and weasels—so the association of armadillo polyembryony with embryonic diapause is probably spurious rather than causal.

A second peculiarity of armadillo pregnancy involves the female's oddly configured uterus, which has only a single blastocyst implantation site (66). One evolutionary hypothesis for how this strange uterine configuration might relate to armadillo polyembryony comes from a surprising juxtaposition between polyembryony in endoparasitic hymenopteran wasps (where the phenomenon is common) and polyembryony in the mammalian genus *Dasypus*.

In the case of the parasitic hymenopterans, a gravid female typically oviposits an egg into the egg of a moth or other host species that she parasitizes. Later, that host egg hatches and grows into a caterpillar that will provide a rich food source for the wasp's offspring that reside within it. The wasp larvae, which have arisen polyembryonically, then eat the host caterpillar from the inside out before pupating on the corpse's skin to continue the endoparasitic life cycle. Thus, for both the endoparasitic hymenopterans and the polyembryonic armadillos, a severe reproductive bottleneck exists such that polyembryonic proliferation might be the best or only available option to augment the size of a clutch. For the wasp, a tiny host egg is the resource constraint that later expands into a spacious caterpillar whose food-rich body can support the development of multiple polyembryonic progeny. For the armadillos, a tiny implantation site is the resource bottleneck that later expands into a spacious intrauterine environment that can house and nourish multiple clonal embryos. Thus, from both the mother's and the offsprings' perspectives, polyembryony might be an evolved way of making the best of the available reproductive situation when a severe but transient limitation exists on offspring numbers within a confined developmental space. This hypothesis is merely a subset of broader evolutionary speculation that polyembryony is genetically favored and tends to evolve whenever offspring in effect have more ecological information about optimal clutch size than do their parents (59). In other words, when progeny are best situated to judge the quality or quantity of environmental resources available to them, they might be under strong selection pressures to adjust the extent of their clonal proliferation accordingly. However, even if such evolutionary speculation has merit, it fails to explain why *Dasypus* armadillos evolved the oddly configured uterine condition in the first place. That mystery remains unsolved.

Clonality via Incest

In theory, consistent self-fertilization (selfing by hermaphroditic individuals) is an intense form of inbreeding with a cascade of population genetic consequences that should include the following: a reduction in effective population size (N_e) compared with bisexual outcrossers; a corresponding diminution of genetic variation at neutral loci; markedly elevated levels of genic homozygosity; a severe reduction in the number of realized recombination events, in effect tightening linkage and heightening gametic-phase disequilibrium throughout the genome, with enhanced opportunities for natural selection to see both beneficial and deleterious mutations and thereby to drive selective sweeps and promote background selection; and a high potential for genetic drift and enhanced opportunities for geographic population structure. Many of these population genetic expectations have been empirically confirmed in various hermaphroditic plants (67, 68) and invertebrate animals (69–71) that regularly engage in facultative self-fertilization.

In the 1960s, Robert Harrington and Klaus Kallman (72–74) reported the discovery of a small hermaphroditic fish (the Mangrove Rivulus, *Rivulus marmoratus*; Cyprinodontidae) that apparently exists in nature as a series of self-fertilizing and highly inbred homozygous clones. In laboratory experiments, these researchers showed that within any clonal strain of this species, artificial fin grafts typically were accepted by recipient fish, thereby indicating a high degree of genetic identity even at histocompatibility loci that otherwise tend to be extremely polymorphic in most fishes and other vertebrates that are sexual outcrossers. Furthermore, artificial fin grafts between some of the inbred lines of *R. marmoratus* were acutely rejected, as might be expected if such isogenic strains often differed from one another genetically.

Across the ensuing decades, the basically clonal nature of *Rivulus* (now *Kryptolebias*) *marmoratus* has been confirmed by genetic studies involving a wide variety of increasingly sophisticated

molecular markers (75–81). Such studies also have disclosed the following further information about *K. marmoratus* (*Kmar*): (*i*) in addition to the self-fertilizing hermaphroditic individuals, pure males exist in some *Kmar* populations (82), thus making this species androdioecious (83), an extremely rare condition in the organic world (84, 85); and (*ii*) these males sometimes mediate outcross events (86, 87), such that the species actually displays a mixed-mating system of predominant selfing with occasional outcrossing (88). Such outcrossing probably happens when a hermaphrodite occasionally sheds a few unfertilized eggs onto which a male then releases sperm (89). Depending on the population, outcrossing rates in *Kmar* are known to vary from a high of about 50% in Belize to a low of less than 5% in Florida. Among all vertebrate animals, members of the Mangrove Rivulus fish clade (90) are unique in displaying each of the following reproductive phenomena: consistent hermaphroditic self-fertilization, androdioecy, and a mixed-mating system (predominant selfing with occasional outcrossing) that routinely yields highly inbred strains that in effect are essentially clonal.

This peculiar suite of biological traits has given rise to a "fireworks model" for the population genetic architecture of *Kmar* at particular geographic sites such as in Florida. Avise (3) explains the fireworks model as follows: "In this metaphor, a black nighttime sky represents the near-complete absence of within-individual heterozygosity in an inbred (highly selfed) population, and each exploding firework represents a single outcross event between distinct homozygous clones. At the core of each explosion is a bright spot of light that represents high heterozygosity in the outcross progeny. Streamers of light, brilliant at first but then quickly fading, burst out of this core as the heterozygous offspring begin to reproduce, often by a resumption of selfing. The many streamers of light that head in different directions represent the many different recombinant genotypes that inevitably arise during this reproductive process, but the streamers fade back into darkness as intrastrain heterozygosity is rapidly lost in each successive generation of selfing. Then another explosion occurs, perhaps in a different part of the nighttime sky, as an outcross event releases another brilliant but temporary burst of genetic variation available for recombination."

Multigenerational self-fertilization is an intense form of inbreeding that at first thought might seem to be highly disadvantageous because of inbreeding depression (diminished survival or fertility) that is expected to arise due to increased homozygosity for deleterious alleles and/or to the loss of fitness-enhancing heterozygosity per se (91). Furthermore, because constitutive self-fertilization in effect stymies the genetic shuffling effects of meiosis and syngamy, each selfing lineage quickly loses genetic variety that otherwise would be available for substantive genetic recombination and potential adaptive responses to changing environments. Accordingly, one traditional argument is that pure selfing tends to be an evolutionary dead end (e.g., refs. 92 and 93; but see also ref. 94). Why then has *Kmar* evolved the propensity to self-fertilize with such regularity? One possibility is that complete selfing simply offers a distinct transmission advantage compared with outcrossing. Within that context, however, low outcrossing levels might be favored because this increases the effective rate of recombination to dissipate negative disequilibria (95). In any event, perhaps some additional clues about the adaptive significance of a mixed-mating system in *Kmar* can be gleaned from various hermaphroditic plants and invertebrate animals that likewise display mixed-mating systems with predominant but not exclusive selfing.

From such mixed-mating taxa, several leading hypotheses for the adaptive significance of selfing (and/or for the evolution of selfing from outcrossing) have been advanced (96). For example, according to Baker (97, 98), the fertilization assurance that selfing automati-

cally affords can be highly advantageous, especially in colonizing species or those that regularly exhibit long distance dispersal in low-density population settings. [This obvious advantage was also well appreciated by Darwin.] By contrast, according to Allard and colleagues (99–103), multilocus coadaptation is a key factor in the success of a self-fertilizing lineage. This latter view, although sometimes criticized [e.g., 104 (pp. 248–249), 105], emphasizes how selfing's restriction on effective genetic recombination can act in conjunction with natural selection to favor the spread of coadapted multilocus suites of alleles that are well molded to local ecological conditions. The argument of Allard and colleagues and Baker's contention are not mutually exclusive, but they do paint very different pictures about the potential ecological benefits and evolutionary significance of consistent self-fertilization in species with mixed-mating systems.

Recently, Avise and Tatarenkov (106) contrasted the argument of Allard and colleagues vs. Baker's contention for the adaptive significance of selfing in *Kryptolebias marmoratus*. Based on the observed patterns of multilocus isogenicity in this species, as well as behavioral and natural history considerations, the authors tentatively concluded that Baker's contention (fertilization assurance) probably offers the better explanation for the evolution and maintenance of self-fertilization in the *Kmar* clade. The Mangrove Rivulus occupies a huge range and inhabits stressful ecological settings prone to disturbance and occasional long-distance dispersal, thus making fertilization assurance an ecological premium for this species. Indeed, *Kmar* is probably a quintessential colonizing species, in which case the ability to self-fertilize (without the need for a sexual partner) is likely to pay reproductive dividends on quite a regular basis in this species.

Discussion

The biological expressions of clonal phenomena in vertebrate animals are highly varied. Some of the clonal processes (such as by artificial nuclear transfer in the laboratory) are human mediated, whereas many others (such as parthenogenesis and polyembryony) occur regularly in nature (as well as under human auspices in some cases). Some of nature's clonal operations (such as parthenogenesis) are intergenerational, whereas others (notably polyembryony) are intragenerational. Some of the clonal proceedings (notably parthenogenesis) are quite strictly asexual, whereas others (such as gynogenesis, hybridogenesis, and kleptogenesis) entail at least some degree of sexuality and sometimes even involve effective genetic recombination to varying extents. Some of the clonal operations apply to whole genomes (parthenogenesis, gynogenesis, and polyembryony), whereas others involve only partial genomes (as in the hemiclones of hybridogens and kleptogens or the partially clonal lineages that arise under mixed-mating systems with predominant selfing). The diverse forms of vertebrate clonality also vary dramatically with respect to their empirical levels of intraindividual heterozygosity: extremely low under constitutive self-fertilization, moderate in magnitude under polyembryony, and extraordinarily high in magnitude under parthenogenesis and its related expressions of unisexuality (due to the interspecific hybrid origins of the typically all-female biotypes).

Although clonality in the vertebrate realm might seem to be a biological aberration, in truth, asexual phenomena are rather widespread in backboned animals, and demonstrably some vertebrate clones or hemiclones have been at least moderately successful on various ecological stages of the evolutionary theater. Furthermore, vertebrate clonality affords novel perspectives on the evolutionary significance of more standard modes of sexual reproduction. Among the many broader evolutionary insights gleaned from clonal or hemiclonal vertebrates can be listed the following: (*i*) interspecific hybridization can in some circumstances give rise to new species (in the form of unisexual biotypes); (*ii*) such speciation events are essentially instantaneous (transpiring

in just a few consecutive animal generations) and they lead to new taxa that are reproductively isolated from one another and from both of their bisexual parental species; (*iii*) most but not all vertebrate clones and clades are evolutionarily short lived (thus giving credence to the standard sentiment that recombinational sex in vertebrate animals is generally important for long-term evolutionary survival); (*iv*) some unisexual lineages actually are quasi-sexual or hemiclonal (thus again hinting that genetic recombination probably has some evolutionary advantages even in the basically asexual realm); (*v*) vertebrate clonality has many varied expressions, some of which are intragenerational, whereas others are intergenerational; and (*vi*) multigeneration inbreeding in effect can also lead to a rare form of quasi-clonal isogenicity, albeit with added evolutionary benefits probably stemming from the retention of occasional outcrossing under a mixed-mating system. Thus, clonal and quasi-clonal vertebrates—despite their relative paucity—have provided some valuable model systems for addressing quite a wide range of ecological and evolutionary topics.

1. Jackson JBC, Buss LW, Cook RE, eds (1985) *Population Biology and Evolution of Clonal Organisms* (Yale Univ Press, New Haven, CT).
2. Avise JC (2004) *The Hope, Hype, and Reality of Genetic Engineering* (Oxford Univ Press, Oxford, UK).
3. Avise JC (2008) *Clonality: The Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals* (Oxford Univ Press, Oxford, UK).
4. Dawley RM, Bogart JP, eds (1989) *Evolution and Ecology of Unisexual Vertebrates* (New York State Museum, Albany, NY).
5. Briggs R, King TJ (1952) Transplantation of living nuclei from blastula cells into enucleated frog's eggs. *Proc Natl Acad Sci USA* 38(5):455–463.
6. Gurdon JB, Laskey RA, Reeves OR (1975) The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs. *J Embryol Exp Morphol* 34(1):93–112.
7. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature* 385(6619):810–813.
8. Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R (1998) Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* 394(6691):369–374.
9. Lee KY, Huang H, Ju B, Yang Z, Lin S (2002) Cloned zebrafish by nuclear transfer from long-term-cultured cells. *Nat Biotechnol* 20(8):795–799.
10. Lee BC, et al. (2005) Dogs cloned from adult somatic cells. *Nature* 436(7051):641–642.
11. Shin T, et al. (2002) A cat cloned by nuclear transplantation. *Nature* 415(6874):859.
12. Betthauser J, et al. (2000) Production of cloned pigs from *in vitro* systems. *Nat Biotechnol* 18(10):1055–1059.
13. Galli C, et al. (2003) Pregnancy: A cloned horse born to its dam twin. *Nature* 424(6949):635.
14. Lanza RP, et al. (2000) Cloning of an endangered species (*Bos gaurus*) using interspecies nuclear transfer. *Cloning* 2(2):79–90.
15. Li Z, et al. (2006) Cloned ferrets produced by somatic cell nuclear transfer. *Dev Biol* 293(2):439–448.
16. Tachibana M, et al. (2013) Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* 153(6):1228–1238.
17. Murphy RW, et al. (2000) A fine line between sex and unisexuality: The phylogenetic constraints on parthenogenesis in lacertid lizards. *Zool J Linn Soc* 130:289–301.
18. Moritz C (1993) The origin and evolution of parthenogenesis in the *Heteronotia binoei* complex: Synthesis. *Genetica* 90:269–280.
19. Sites JW, Peccinini-Seale DM, Moritz C, Wright JW, Brown WM (1990) The evolutionary history of parthenogenetic *Cnemidophorus lemniscatus* (Sauria, Teiidae). I. Evidence for a hybrid origin. *Evolution* 44:906–921.
20. Adams M, Foster R, Hutchinson MN, Hutchinson RG, Donnellan SC (2003) The Australian scincid lizard *Menetia greyii*: A new instance of widespread vertebrate parthenogenesis. *Evolution* 57(11):2619–2627.
21. Wynn AL, Cole CJ, Gardner AL (1987) Apparent triploidy in the unisexual Brahminy blind snake *Rhamphotyphlops braminus*. *Am Mus Novit* 2868:1–7.
22. Groot TVM, Bruins E, Breeuwer JAJ (2003) Molecular genetic evidence for parthenogenesis in the Burmese python, *Python molurus bivittatus*. *Heredity (Edinb)* 90(2):130–135.
23. Chapman DD, et al. (2007) Virgin birth in a hammerhead shark. *Biol Lett* 3(4):425–427.
24. Dickinson TA, Phipps JB (1986) The breeding system of *Crataegus crus-galli sensu lato* in Ontario (Canada). *Am J Bot* 73:116–130.
25. Avise JC, Trexler JC, Travis J, Nelson WS (1991) *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. *Evolution* 45(6):1530–1533.
26. Echelle AA, Echelle AF (1997) Patterns of abundance and distribution among members of a unisexual-bisexual complex of fishes (Atherinidae: *Menidia*). *Copeia* 1997:249–259.
27. Dawley RM, Schultz RJ, Goddard KA (1987) Clonal reproduction and polyploidy in unisexual hybrids of *Phoxinus eos* and *Phoxinus neogaeus* (Pisces: Cyprinidae). *Copeia* 1987:275–283.
28. Janko K, et al. (1997) The gynogenetic reproduction of diploid and triploid hybrid spined loaches (*Cobitis*: Teleostei), and their ability to establish successful clonal lineages—On the evolution of polyploidy in asexual vertebrates. *Genetica* 131:185–194.
29. Dawley RM (1992) Clonal hybrids of the common laboratory fish *Fundulus heteroclitus*. *Proc Natl Acad Sci USA* 89(6):2485–2488.
30. Uzzell T (1964) Relations of the diploid and triploid species of the *Ambystoma jeffersonianum* complex (Amphibia, Caudata). *Copeia* 1964:257–300.
31. Schultz RJ (1969) Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *Am Nat* 103:606–619.

32. Carmona JA, Sanjur OI, Doadrio I, Machordom A, Vrijenhoek RC (1997) Hybridogenetic reproduction and maternal ancestry of polyploid Iberian fish: The *Tropidophoxinellus alburnoides* complex. *Genetics* 146(3):983–993.
33. Vrijenhoek RC, Dawley RM, Cole CJ, Bogart JP (1989) *Evolution and Ecology of Unisexual Vertebrates*, eds Dawley RM, Bogart JP (New York State Museum, Albany, NY), pp 19–23.
34. Uzzell TM, Berger L (1975) Electrophoretic phenotypes of *Rana ridibunda, Rana lessonae*, and their hybridogenetic associate *Rana esculenta*. *Proc Natl Acad Sci USA* 127:13–24.
35. Bi K, Bogart JP (2006) Identification of intergenomic recombinations in unisexual salamanders of the genus *Ambystoma* by genomic *in situ* hybridization (GISH). *Cytogenet Genome Res* 112(3-4):307–312.
36. Uzzell T, Günther R, Berger L (1977) *Rana ridibunda* and *Rana esculenta*: A leaky hybridogenetic system (Amphibia, Salientia). *Proc Acad Nat Sci Phila* 128:147–171.
37. Bogart JP, Bi K, Fu J, Noble DWA, Niedzwiecki J (2007) Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. *Genome* 50(2):119–136.
38. Moritz C, et al. (1992) The maternal ancestry and approximate age of parthenogenetic species of Caucasian rock lizards (*Lacerta*: Lacertidae). *Genetica* 87:53–62.
39. Uzzell TM (1970) Meiotic mechanisms of naturally occurring unisexual vertebrates. *Am Nat* 104:433–445.
40. Moritz CC, Donnellan S, Adams M, Baverstock PR (1989) The origin and evolution of parthenogenesis in *Heteronotia binoei* (Gekkonidae): Extensive genotypic diversity among parthenogens. *Evolution* 43:994–1003.
41. Quattro JM, Avise JC, Vrijenhoek RC (1992) Mode of origin and sources of genotypic diversity in triploid gynogenetic fish clones (Poeciliopsis: Poeciliidae). *Genetics* 130(3):621–628.
42. Alves MJ, Coelho MM, Próspero MI, Collares-Pereira MJ (1999) Production of fertile unreduced sperm by hybrid males of the Rutilus alburnoides complex (Teleostei, cyprinidae). An alternative route to genome tetraploidization in unisexuals. *Genetics* 151(1):277–283.
43. Choleva L, et al. (2012) Synthesis of clonality and polyploidy in vertebrate animals by hybridization between two sexual species. *Evolution* 66(7):2191–2203.
44. Cuellar O (1974) On the origin of parthenogenesis in vertebrates: The cytogenetic factors. *Am Nat* 108:625–648.
45. Cuellar O (1977) Animal parthenogenesis. *Science* 197(4306):837–843.
46. Schultz RJ (1969) Hybridization, unisexuality and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *Am Nat* 103:605–619.
47. Densmore DD, Moritz CC, Wright JW, Brown WM (1989) Mitochondrial-DNA analyses and the origin and relative age of parthenogenetic lizards (genus *Cnemidophorus*). IV. Nine *sexlineatus*-group unisexuals. *Evolution* 43:969–983.
48. Bernstein C, Bernstein H (1991) *Aging, Sex, and DNA Repair* (Academic Press, New York).
49. Muller HJ (1964) The relation of mutation to mutational advance. *Mutat Res* 106:2–9.
50. Vrijenhoek RC (1984) *Evolutionary Genetics of Fishes*, ed Turner BJ (Springer-Verlag, New York), pp 399–429.
51. Vrijenhoek RC (1979) Factors affecting clonal diversity and coexistence. *Am Zool* 19:787–797.
52. Avise JC, Quattro JM, Vrijenhoek RC (1992) Molecular clones within organismal clones: mitochondrial DNA phylogenies and the evolutionary histories of unisexual vertebrates. *Evol Biol* 26:225–246.
53. Spolsky CM, Phillips CA, Uzzell T (1992) Antiquity of clonal salamander lineages revealed by mitochondrial DNA. *Nature* 356(6371):706–708.
54. Quattro JM, Avise JC, Vrijenhoek RC (1992) An ancient clonal lineage in the fish genus *Poeciliopsis* (Atheriniformes: Poeciliidae). *Proc Natl Acad Sci USA* 89(1):348–352.
55. Maynard Smith J (1992) Age and the unisexual lineage. *Nature* 356:661–662.
56. Godfray HJC (1994) *Parasitoids: Behavioral and Evolutionary Ecology* (Princeton Univ Press, Princeton).
57. Richards AJ, ed (1997) *Plant Breeding Systems* (Chapman and Hall, New York).
58. Craig SF, Slobodkin LB, Wray G (1995) The 'paradox' of polyembryony. *Trends Ecol Evol* 10(9):371–372.
59. Craig SF, Slobodkin LB, Wray GA, Biermann CH (1997) The "paradox" of polyembryony: A review of the cases and a hypothesis for its evolution. *Evol Ecol* 11:127–143.
60. Williams GC (1975) *Sex and Evolution* (Princeton Univ Press, Princeton).
61. Loughry WJ, Prodöhl PA, McDonough CM, Avise JC (1998a) Polembryony in armadillos. *Am Sci* 86:274–279.

62. Prodöhl PA, Loughry WJ, McDonough CM, Nelson WS, Avise JC (1996) Molecular documentation of polyembryony and the micro-spatial dispersion of clonal sibships in the nine-banded armadillo, Dasypus novemcinctus. *Proc Biol Sci* 263(1377): 1643–1649.

63. Billingham RE, Neaves WB (1980) Exchange of skin grafts among monozygotic quadruplets in armadillos. *J Exp Zool* 213(2):257–260.

64. Loughry WJ, Dwyer GM, McDonald CM (1998b) Behavioral interactions between juvenile nine-banded armadillos (*Dasypus novemvinctus*) in staged encounters. *Am Midl Nat* 139:125–132.

65. Stockard CR (1921) A probable explanation of polyembryony in the armadillo. *Am Nat* 55:62–68.

66. Galbreath GJ (1985) *The Evolution of Armadillos, Sloths, and Vermilinguas*, ed Montgomery GG (Smithsonian Press, Washington, DC), pp 243–246.

67. Hamrick JL, Godt MJ (1996) Effects of life history traits on genetic diversity in plant species. *Philos Trans R Soc Lond, B* 351:1291–1298.

68. Charlesworth D (2003) Effects of inbreeding on the genetic diversity of populations. *Philos Trans R Soc Lond B Biol Sci* 358(1434):1051–1070.

69. Jarne P (1995) Mating systems, bottlenecks and genetic polymorphism in hermaphroditic animals. *Genet Res* 65:193–207.

70. Cutter AD (2006) Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer Caenorhabditis elegans. *Genetics* 172(1):171–184.

71. Graustein A, Gaspar JM, Walters JR, Palopoli MF (2002) Levels of DNA polymorphism vary with mating system in the nematode genus caenorhabditis. *Genetics* 161(1): 99–107.

72. Harrington RW, Jr (1961) Oviparous hermaphroditic fish with internal self-fertilization. *Science* 134(3492):1749–1750.

73. Kallman KD, Harrington RW, Jr (1964) Evidence for the existence of homozygous clones in the self-fertilizing hermaphroditic teleost Rivulus marmoratus (Poey). *Biol Bull* 126:101–114.

74. Harrington RW, Jr, Kallman KD (1968) The homozygosity of clones of the self-fertilizing hermaphroditic fish Rivulus marmoratus Poey (Cyprinodontidae, Atheriniformes. *Am Nat* 102:337–343.

75. Massaro EJ, Massaro JC, Harrington RE, Jr (1975) *Isozymes*, ed Markert CL (Academic Press, NY), Vol 3, pp 439–453.

76. Vrijenhoek RC (1985) Homozygosity and interstrain variation in the self-fertilizing hermaphroditic fish, Rivulus marmoratus. *J Hered* 76:1475–1486.

77. Turner BJ, Elder JF, Jr, Laughlin TF, Davis WP (1990) Genetic variation in clonal vertebrates detected by simple-sequence DNA fingerprinting. *Proc Natl Acad Sci USA* 87(15):5653–5657.

78. Weibel AC, Dowling TE, Turner BJ (1999) Evidence that an outcrossing population is a derived lineage in a hermaphroditic fish (*Rivulus marmoratus*). *Evolution* 53: 1217–1225.

79. Sato A, et al. (2002) Persistence of *Mhc* heterozygosity in homozygous clonal killifish, *Rivulus marmoratus*: Implications for the origin of hermaphroditism. *Genetics* 162(4):1791–1803.

80. Mackiewicz M, et al. (2006a) Microsatellite documentation of male-mediated outcrossing between inbred laboratory strains of the self-fertilizing mangrove killifish (*Kryptolebias Marmoratus*). *J Hered* 97(5):508–513.

81. Tatarenkov A, et al. (2007) Strong population structure despite evidence of recent migration in a selfing hermaphroditic vertebrate, the mangrove killifish (*Kryptolebias marmoratus*). *Mol Ecol* 16(13):2701–2711.

82. Turner BJ, Davis WP, Taylor DS (1992a) Abundant males in populations of a selfing hermaphroditic fish—*Rivulus marmoratus*—from some Belize cays. *J Fish Biol* 40(2): 307–310.

83. Mackiewicz M, Tatarenkov A, Taylor DS, Turner BJ, Avise JC (2006b) Extensive outcrossing and androdioecy in a vertebrate species that otherwise reproduces as a self-fertilizing hermaphrodite. *Proc Natl Acad Sci USA* 103(26):9924–9928.

84. Pannell JR (2002) The evolution and maintenance of androdioecy. *Annu Rev Ecol Syst* 33:397–425.

85. Avise JC (2011) *Hermaphroditism: A Primer on the Biology, Ecology, and Evolution of Dual Sexuality* (Columbia University Press, New York).

86. Lubinski BA, Davis WP, Taylor DS, Turner BJ (1995) Outcrossing in a natural population of a self-fertilizing hermaphroditic fish. *J Hered* 86:469–473.

87. Taylor DS, Fisher MT, Turner BJ (2001) Homozygosity and heterozygosity in three populations of *Rivulus marmoratus*. *Environ Biol Fishes* 61:455–459.

88. Mackiewicz M, Tatarenkov A, Turner BJ, Avise JC (2006c) A mixed-mating strategy in a hermaphroditic vertebrate. *Proc Biol Sci* 273(1600):2449–2452.

89. Harrington RW, Jr (1963) Twenty-four hour rhythms of internal self-fertilization and of oviposition by hermaphrodites of *Rivulus marmoratus*. *Physiol Zool* 36:325–331.

90. Tatarenkov A, Lima SMQ, Taylor DS, Avise JC (2009) Long-term retention of self-fertilization in a fish clade. *Proc Natl Acad Sci USA* 106(34):14456–14459.

91. Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Syst* 18:237–268.

92. Stebbins GL (1957) Self fertilization and population variability in higher plants. *Am Nat* 91:337–254.

93. Grant V (1958) The regulation of recombination in plants. *Cold Spring Harb Symp Quant Biol* 23:337–363.

94. Igic B, Busch JW (2013) Is self-fertilization an evolutionary dead end? *New Phytol* 198(2):386–397.

95. Kamran-Disfani A, Agrawal AF (2014) Selfing, adaptation and background selection in finite populations. *J Evol Biol* 27(7):1360–1371.

96. Busch JW, Delph LF (2012) The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Ann Bot (Lond)* 109(3):553–562.

97. Baker HG (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evolution* 9:347–349.

98. Baker HG (1967) Support for Baker's Law as a rule. *Evolution* 21:853–856.

99. Allard RW (1975) The mating system and microevolution. *Genetics* 79(Suppl): 115–126.

100. Allard RW, Babbel GR, Clegg MT, Kahler AL (1972) Evidence for coadaptation in *Avena barbata*. *Proc Natl Acad Sci USA* 69(10):3043–3048.

101. Clegg MT, Allard RW (1972) Patterns of genetic differentiation in the slender wild oat species *Avena barbata*. *Proc Natl Acad Sci USA* 69(7):1820–1824.

102. Clegg MT, Allard RW, Kahler AL (1972) Is the gene the unit of selection? Evidence from two experimental plant populations. *Proc Natl Acad Sci USA* 69(9):2474–2478.

103. Hamrick JL, Allard RW (1972) Microgeographical variation in allozyme frequencies in *Avena barbata*. *Proc Natl Acad Sci USA* 69(8):2100–2104.

104. Lewontin RC (1974) *The Genetic Basis of Evolutionary Change* (Columbia Univ Press, New York).

105. Latta RG (2009) Testing for local adaptation in *Avena barbata*: A classic example of ecotypic divergence. *Mol Ecol* 18(18):3781–3791.

106. Avise JC, Tatarenkov A (2012) Allard's argument versus Baker's contention for the adaptive significance of selfing in a hermaphroditic fish. *Proc Natl Acad Sci USA* 109(46):18862–18867.