

Concept Paper

# Why Self-fertilizing Plants Still Exist in Wild Populations: Diversity Assurance through Stress-Induced Male Sterility May Promote Selective Outcrossing and Recombination

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**Abstract:** Climate change creates challenges for wild species, but plants have survived and adapted to similar changes in their evolutionary past. Most plants were originally outcrossing, one theoretical genetic reason being that self-fertilization does not create novel recombinants that allow adaptation. Thus selfing seems an evolutionary “dead end”. Nevertheless, self-fertilizing plants make up 14% of seed plant species. We offer a new interpretation of a response by self-fertilizing wild species to extreme existential threats, which creates novel recombinant progeny. This proposed mechanism goes beyond reproductive assurance, the usual explanation of selfing. Extreme stress, such as excessive heat within a specific window, first makes plants male-sterile, while female organs remain functional and can receive wind-borne pollen from any of the few nearby stress-tolerant individuals. Thus stress-induced male sterility enables and/or enhances outcrossing in selfing plants. Although in practice this proposed mechanism requires very special circumstances and operates only in certain species with conducive floral traits, we posit that over evolutionary time even such rare events can make a significant lasting impact on a species’ survival in changing conditions. This proposed mechanism, which we call Diversity Assurance, allows a population subject to severe stress to sample preferentially those genes that underpin tolerance to that specific stress. These genes are then recombined in subsequent generations, along with the male-sterility-under-stress trait of the female parent. This contributes in part to explain the effective evolution and hence persistence of self-fertilizing species. Diversity Assurance, we propose, is an adaptive mechanism that has been selected under extreme stress, underpinned by a simple loss-of-function of the male reproductive system. It may be triggered not only by heat, but also by other stressors. This proposed mechanism helps to explain why even highly self-fertilizing plant species remain able to respond to environmental changes through triggered outcrossing.

**Keywords:** self-fertilization; cross-pollination; genetic diversity; adaptation; climate change

## 1. Introduction

Climate change and its impacts have been well documented [1]. Among other factors, climate change results in greater variation in the frequency and intensity of extreme weather events, such as wind, temperature, precipitation, and light intensity. While climate change is clearly accelerating now, its effects have occurred many times before over the Earth’s history, and individuals of many species

have surely retained some ability to survive such stresses [2]. Short-term survival of organisms—*sine qua non* for long-term adaptive changes—is much more dependent on dealing with abiotic extremes than with changes in average weather. Seed plants, which are sessile and cannot move out of harm's way, are especially exposed to these extremes. How did they and their ancestors survive such stresses in the past?

This paper identifies a rare but radical type of natural selection that certain plants may resort to at times of existential abiotic stress during reproduction, in which those genes that provide increased tolerance to that specific stress can be sampled, recombined and increased in frequency.

## 2. Abiotic Stress on Reproduction

While many abiotic stresses may affect plant reproduction, the negative effect of heat is perhaps the most studied. Male reproduction systems tend to be more sensitive to heat stress than are female reproductive systems, but there are exceptions [3–14]. For most plants, temperatures above 30 °C are often fatal for pollen [6,10]. Even the wild coyote gourd (*Cucurbita palmata*), which grows and survives in the hot deserts of southwest USA at temperatures higher than 45 °C, at 36 °C becomes 90% male sterile [10]. The associated damage can involve a variety of processes, such as impaired meiosis, reactive oxygen species (ROS) regulatory systems, microsporogenesis, microgametogenesis, tapetum development, anthesis, dehiscence, pollen adhesion on the stigma, pollen germination, and fertilization [10,11,15–19]. As stress increases, the female reproductive system is also eventually compromised and no longer functions. But there is a window during which the male system shuts down but the female system is still operational. For chill stress to winter wheat, the female system remained unaffected by a drop in night temperature of 13.5 °C (from 15 °C to 1.5 °C), while the male system became sterile [4]. For heat stress to chickpea, the female system was still receptive at 40.2 °C, while the male system became 50% sterile at 36 °C [5]. In another study of chickpea, the female system was still active at 45 °C [7]. For heat stress in pea, the female system was still more than 90% active at 35 °C, while pollen viability had dropped below 40% [8]. For heat stress in *Arabidopsis*, the female system remained fully functional at 35 °C, when the male system had lost all viability [14].

The concept advanced in this paper, which we call Diversity Assurance, builds on the extensive literature on the effect of heat on pollen development. However, many of the implications that follow hold equally for the male-sterilizing effects of cold, drought and other stresses on seed plants [10,11,15,20–22]. Müller and Rieu proposed a similar idea based on the high temperature response of pollen, but did not expand on it or underpin it with a body of supportive evidence and reasoning [23]. We do so in subsequent sections.

## 3. Evolution of Mating Systems: From Outcrossing to Selfing

In the history of the plant kingdom, it is common to see transitions from cross-fertilization (outcrossing, allogamy) to self-fertilization (selfing, autogamy), partly because selfing provides reproductive assurance when pollinators or mates are rare [24,25]. However, Stebbins described selfing as a “dead end of evolution” [26]. Selfing is assumed to eventually lead to extinction due to decreased effectiveness of recombination and reduced genetic diversity. Stebbins further asserted that the route from outcrossing self-incompatibility to selfing self-compatibility, a “derived condition”, is very much irreversible [26]. Once compromised, the various complex, multi-component, genetic systems that underpin outcrossing are unlikely to be regained. Selfing does indeed reduce the gene pool, or, more precisely, the number of distinct alleles, and consequently the expected rate of adaptation to change [25]. Selfing then purges deleterious, recessive genes, thereby reducing inbreeding depression [25,27].

Despite Stebbins' “chronicle of a death foretold”, about 14% of all seed-producing plant species are predominantly to strongly selfing [28]. Reasons for their continued success include higher reproductive rates (i.e., *r*-strategy), higher mutation rates, and less selfish genetic elements [29]. Modern phylogenetic modeling has also called their predicted demise into doubt [30].

#### 4. Mixed-Mating Systems: Having It Both Ways

Despite Stebbins' theory that plants would be driven to either one of the two evolutionary stable mating systems—complete selfing or complete outcrossing—about 42% of 345 seed plant species express an intermediate, mixed-mating system, combining the abilities to self and outcross with perfect flowers that contain both male and female organs [28,31]. In a recent study of 741 populations of 105 species, mixed-mating was present in 63% of the species [32]. Stress of various kinds can lead to one mode of reproduction, selfing or outcrossing, being favored, and this plasticity is considered one of the main reasons for the wide presence of mixed-mating systems. When mates or pollinators are scarce and thus limit outcrossing, selfing allows for easy reproductive assurance. On the other hand, when selfing is limited, as it might be under conditions of heat stress, for example, pollen vectors may facilitate more cross pollination, and thus reproductive assurance is obtained through outcrossing.

In the search for additional reproductive assurance in mixed-mating systems, we can even add vegetative reproduction to the mix. *Trifolium polymorphum*, for example, is a forage component of natural pastures in South America that combines cross-fertilization, self-fertilization and vegetative reproduction [33]. This mixed-mating reproductive system has aerial flowers that are mostly cross-pollinated by pollinators. However, pollinator movements may also facilitate delayed self-compatible selfing as pollen is dislodged within the flower. In addition, *T. polymorphum* has subterranean cleistogamous flowers that must self-fertilize. As a further protection against the loss of propagules to herbivory, *T. polymorphum* also produces clonal subterranean stolons. Truly, a plant for all seasons.

#### 5. Reproductive Assurance in Mixed-Mating by Stress-Induced Selfing

In a survey of 80 mixed-mating species from 38 families with facultative self-fertilization of flowers, delayed selfing was the mode to reproductive assurance in all cases where information on the timing of selfing was known [34]. Delayed selfing is clearly a rescue mechanism in case cross-pollination through pollinators had not been achieved near the end of the flower's lifespan. Studies on when, during their lifespan, mixed-mating plants move from outcrossing to self-fertilization indicate that they adaptively track fitness optima determined by, for example, pollinator presence and activity [35]. These studies provide a deeper understanding of this move from outcrossing to selfing.

*Sebaea* (Gentianaceae) has a stigma at the top of the style and a secondary stigma underneath it, halfway down the style, an arrangement called diplostigmaty [36]. The secondary stigma allows for autonomous delayed self-pollination by pollen falling from the same flower's anthers onto its lower stigma. Diplostigmaty permits this otherwise mostly insect pollinated species to achieve reproductive assurance under the stress of either sparse available mates or low pollinator numbers.

Other mixed-mating species show a variety of complementary reproductive systems. *Gymnarrhena micrantha* (Asteraceae), found in North Africa and the Middle East, produces aerial, dioecious, chasmogamous flowers with anthers and stigma exposed, and subterranean homogamous, cleistogamous, closed flowers [37]. The above-ground flowers are functionally dioecious with separate female and male heads, encouraging outcrossing and recombination, and produce wind-dispersed seeds. The underground flowers are cleistogamous, ensuring self-fertilization, with the seed germinating in the same, apparently favorable, location in which the mother plant is or was growing. The below-ground selfing inflorescences flower and form fruits first, even in very dry years. The above-ground outcrossing inflorescences flower and produce fruits later in the season, and only in wet years. Again, selfing provides reproductive assurance in the stress of dry seasons.

Four populations of the annual weedy species *Hypochaeris salzmanniana* (Asteraceae) occupy the coastal sandy regions in southern Spain, from west to east; their reproductive behavior is governed by varying degrees of self-incompatibility [38]. Increasing winds along the coastal gradient in an easterly direction mean that the pollinators (mostly bees) that effect cross-pollination are most active in the west and decreasingly so moving east. The most easterly plant populations express lower inbreeding depression and higher levels of self-compatibility, and give rise to progeny that are mostly

self-compatible. It would appear that in the east, where pollinator activity was lower and more unpredictable as a result of the abiotic stress of strong winds, increased levels of self-compatibility ensure reproductive assurance through selfing [38,39]. Such plasticity is even more advantageous when species are annual and may need to reproduce every flowering season [38]. Thus, reproductively mixed populations can be stable and responsive under varying conditions. Arista et al., also speculated that self-compatibility followed by selfing is promoted when the amount of outcross or compatible pollen is limited due to stressed conditions, as again selfing offers reproductive assurance [38].

## 6. Reproductive Assurance in Mixed-Mating by Stress-Induced Outcrossing

Most of the literature on mixed-mating systems that respond to stress by adjusting their balance of reproductive options is on cross-fertilizing, self-incompatible species that maintain some self-fertilizing, self-compatible ability, which contributes to reproductive assurance [28]. A further consideration is that cleistogamous flowers are often smaller and more energy-efficient than are chasmogamous, larger flowers, making them a more resource-effective fall-back when outcrossing lags [40]. Overall, the argument that these are adaptations that favor reproductive assurance is logical and biologically feasible.

But what about the opposite: mixed-mating plants that are mostly self-fertile and that occasionally engage in cross-fertilization for reasons of reproductive assurance? If selfing species evolved from outcrossing ancestors, and if the fully-functioning self-incompatibility apparatus once lost is difficult to re-assemble from scratch, as experts argue, then maintaining some ability to outcross for reproductive assurance, especially in times of stress, would appear to have selective advantage.

Indeed, many such cases exist, most involving pollinator-facilitated outcrossing. Even when cross-pollination by pollinators becomes less essential for the reproductive success of the plant, considerations of mutualism argue for the maintenance of outcrossing. The “persistent pollinator” hypothesis posits that even though the plant may have evolved to be more independent of the pollinator, the pollinator may not have evolved, and remains highly reliant on nectar and pollen from the plant. In the process of harvesting these, it will bring about outcrossing, even when inbreeding depression is low [31].

Within all angiosperms, near-cleistogamy and hence selfing is particularly common among the grasses [40–42]. However, these grasses typically also produce chasmogamous, open-pollinated flowers that allow outcrossing [40,43,44]. While the assumption is that selfing provides reproductive assurance under conditions of stress, this proves to be not always, or even mostly, the case. In fact, the reasons for this plasticity in reproductive system, and the balance it strikes between selfing and outcrossing are neither straightforward nor well understood. The logic that selfing through cleistogamy is most energy-efficient in securing progeny only applies to some species, while in other cases, the opposite is observed [40]. Some species that are mostly self-pollinating also allow pollination by insects, which generally results in outcrossing. When the amount of selfing drops due to stress, attracting more pollinators can help achieve reproductive assurance through outcrossing [45].

Faba bean (*Vicia faba*) (Fabaceae) is normally self-fertilized for 75% of its seed set, while the remainder is “pollinator-facilitated”; that is, pollen may be brought from flowers on the same plant, which would result in selfing, or from a separate plant, which would result in outcrossing [45]. Heat stress, artificially achieved by raising the daily temperature from 26 °C to 34 °C for five days, resulted in reduced selfing and seed set. However, when bumblebee pollinators were abundant, seed set increased from 16% to 53%. However, it was not clear whether the bumblebees had actually brought about outcrossing, or whether their visits, which are associated with stamens being shaken and pollen launched within the flower, had facilitated selfing. In order to distinguish these possibilities, in a subsequent experiment, Bishop et al., used hilum color to distinguish between selfed and outcrossed seed [46]. The proportion of outcrossed seeds following five days of artificial heat stress (20 °C to 30 °C during day) due to the pollinating bumblebees increased from 17% to 33%. Clearly, heat had reduced selfing, which pollinator-assisted outcrossing could correct.

*Silene noctiflora* (Caryophyllaceae) is a gynomonoecious annual that produces both chasmogamous, perfect (hermaphrodite) flowers with female pistils and male stamens that are in close contact with the styles and fully female (pistillate) flowers with longer styles that reach beyond the flower opening and thus facilitate outcrossing [47]. The corollas of the perfect flowers open only for two successive nights, while pistillate flowers are open day and night for the lifespan of the flower, if not pollinated. The perfect flowers are fully self-compatible, and given the proximity of the female and male structures are self-pollinated in 95% of the cases; the remainder outcross, mostly by moths [47,48]. In principle, the pistillate (female) flowers can be selfed by pollen from perfect flowers nearby on the same plant, but are mostly outcrossed with pollen from other plants, also conveyed by moths. When the daily temperature was raised from 19 °C to 25 °C, the proportion of pistillate flowers increased from 3.2% to 9.3% of all flowers on the plant, thus enhancing the femaleness of the plant. In addition to increasing the proportion of pistillate flowers, elevated temperatures also increased the presence of pollinating moths, especially during the night. As a result, outcrossing increased. Folke and Delph speculated that *Silene noctiflora* may have evolved to produce more female flowers when temperatures rise, because that is when there are more pollinators available for successful production of outcrossed seed, again offering reproductive assurance [47].

*Clarkia concinna* and *C. breweri* (Onagraceae) are usually outcrossed by insect pollinators [49]. Under drought stress, *C. concinna* self-pollinated more, which the authors predicted would occur for reasons of reproductive assurance. However, the sister species, *C. breweri*, showed the opposite behavior, outcrossing more under drought stress, which had not been predicted. This difference between the two species presented the authors with an unexpected conundrum. They noted that: "... it may be that autogamy decreases with drought if there is strong selection to outcross under drought conditions." and "Our results show that environmental stress does not necessarily lead to higher rates of autogamy, as has previously been hypothesized." [49], but did not elaborate these thoughts further.

There is some evidence that species potentially under attack from several fungal pathogens express higher rates of outcrossing than do species with fewer such enemies [50]. Levin had suggested this idea of an "open recombination system" already in 1975, based on the notion that outcrossed progeny would express new recombined phenotypes that would convey improved resistance to intense, systematic pressure from pathogens and pests, and hence greater evolutionary fitness [51]. Such a mechanism could also drive the continued presence of a minimum low level of outcrossing in predominantly selfing species, to further their potential adaptation to co-evolving pathogens.

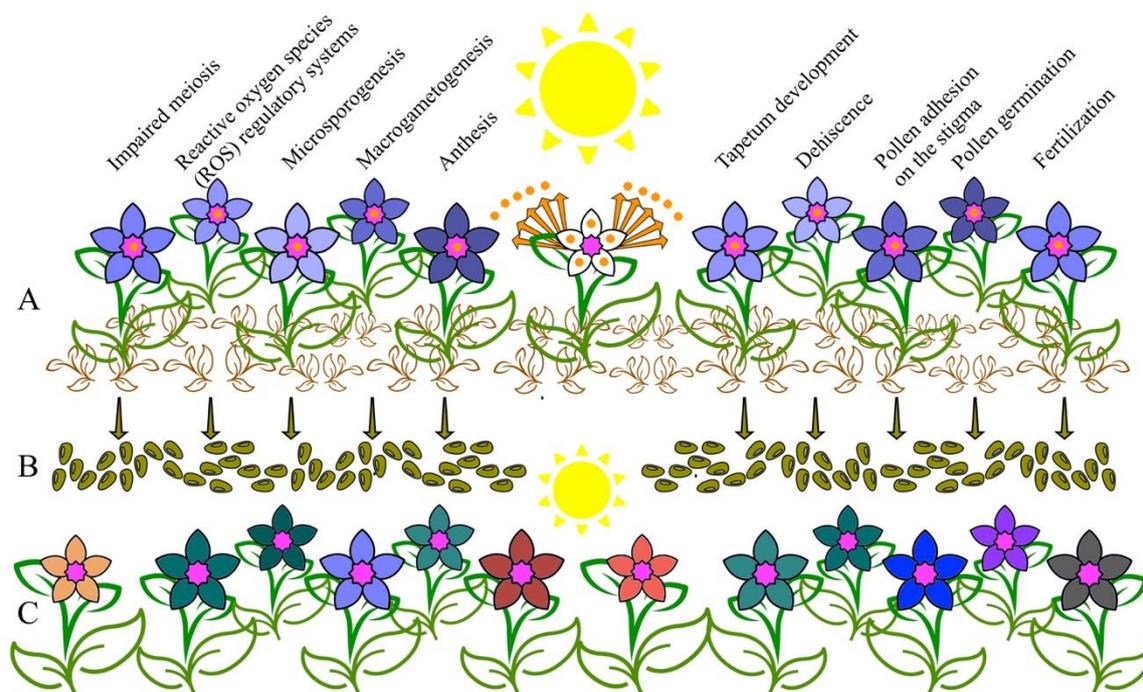
## 7. Diversity Assurance: Stress-induced Male Sterility Promotes Outcrossing

Some categories of plant are very highly selfing. They often have very simple, small, highly energy-efficient, (near-) cleistogamous flowers that do not attract pollinators. They express no inbreeding depression and can be highly productive. Several selfing taxa contain apomictic species that do not require active pollen to produce progeny, and hence whose reproduction is not hindered by pollen sterility. Here we focus on those self-fertilizing wild species that do not have such an apomictic back-up strategy [26,52,53].

There is another way for such highly selfing plants to achieve outcrossing, not through showy flowers but through inherited sensitivity to existential threats: stress-induced male sterility, which completely disables selfing. Corrupting any essential morphological, structural or metabolic process in male fertility will do the job; hence the many reported causes of male sterility, from early-stage impaired microsporogenesis to late-stage failed pollen adhesion on the stigma. Before stress fully incapacitates the plant during this window of opportunity, the female reproductive organs on stress-induced male-sterile plants remain functional and can receive wind-borne pollen from other stress-tolerant plants that are still male-fertile, effectively outcrossing. We refer above to some examples in which first the male and then the female reproductive systems become disabled, and although of limited duration, this window seems to be quite well defined in those studies.

Stigmas need to be accessible to outside pollen for pollination to take place, and in very tightly cleistogamous species such access is impossible. In other cases, access will be possible as a result of floral morphology. In a few cases, such as some wheat and barley varieties and possibly some of their wild relatives, the flower offers a “second opening” after a few days of not being (self-) fertilized [54]. No published information seems available on whether this “second opening” response occurs in wild plant populations. Our proposed mechanism would be restricted only to those selfing species where access for outside pollen to the stigma is possible. Thus stress-induced male sterility can enable or enhance wind-facilitated outcrossing in certain selfing plants.

As indicated, the environmental conditions required are special, and the kind of plant species appropriate for this mode of enhancing recombination must have conducive floral traits. However, even if rare, we posit that over the evolutionary span of these specific selfing species in conditions of existential threat, the Diversity Assurance mechanism can play a role in generating novel recombinants (Figure 1). Wild species, which are not grown as monocultures (unlike most crops), will have greater average distances between individual plants of the same wild species. Hence any form of cross-pollination by wind will require pollen to travel over these greater distances.



**Figure 1.** Conceptual model of Diversity Assurance. (A) Under excess heat, most plants die without flowering. A few (colored) survive as female only plants, rendered male sterile by one or more of at least 10 mechanisms that are known to affect pollen development and fertilization. Tolerance to these effects allows even fewer plants (white) to produce pollen (orange), which can then cross-fertilize through wind-borne pollen any of the male-sterile plants. (B) The cross-fertilized male-sterile flowers (colored) produce fertile seeds, which give rise to a new generation. (C) The new generation has enhanced diversity and stress-tolerance, because recombinant offspring carry stress tolerance from both parents and the male-sterility-under-stress trait of the female parent. Diversity Assurance events occur again in subsequent generations with new rounds of recombination, as result of which additional new combinations of stacked stress-tolerance genes will be created that will be open to selection.

This paper addresses the situation in wild populations, but few if any studies have been carried out on stress-induced male sterility leading to increased outcrossing in such natural selfing plant communities [55]. Hence we list here some relevant published research on outcrossing as a result of stress-induced male sterility in highly selfing crops. In, for example, common bean, chickpea,

oilseed rape, pea, tomato and wheat, plants rendered male sterile by heat stress were able to set seed when viable external pollen was provided [5,8,46,56]. Increased outcrossing was shown to express varietal differences in drought-stressed and cold-stressed male-sterile wheat [57,58]. In precise phytotron and field studies, Weerakoon et al., showed why the occurrence of outcrossing of male-sterile plants in selfing crops such as rice is underestimated; often the outcrossed pollen originates from adjacent plants of the same cultivar, and hence no obvious spike sterility or subsequent segregation is observed [59]. This would partially explain why outcrossing due to stress-induced male-sterility is likely underreported in selfing species, including wild populations. Paradoxically, the very existence and effectiveness of diversity assurance may obscure its detection.

Rather than requiring a multitude of gains-of-function, this proposed mechanism is based on a simple loss-of-function of the male reproductive system. It allows these plants, now functioning only as females, because they are still stress-tolerant enough to maintain operational female reproductive mechanisms, to greatly increase the genetic diversity captured in their outcrossed seeds from any suitably close same-species neighbors that are sufficiently stress-tolerant to produce pollen, which is then transported by the wind. The contributing parents may well have different genes that confer heat tolerance, operating at the vegetative and reproductive level. Although these are wild selfing populations, studies have shown that within-population genetic diversity can be moderate and significant in some of them, and as such they are heterogeneous populations of near-homozygous individual plants. This has been documented in diverse wild populations of grasses, including wild oats in China and the USA [60], *Elymus breviaristatus* in China [61], *Elymus nutans* and *Elymus sibiricus*, important perennial forages, in China [62], *Elymus tangutorum*, a pasture bunchgrass, in China [63], wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) in Israel [64], *Thinopyrum junceum* and *Thinopyrum junceiforme* in Spain [65], *Paspalum dilatatum* subsp. *flavescens*, a perennial forage, in Uruguay [66], and the crucifer, *Thlaspi arvense* L., field pennycress, in the USA [67].

Subsequent repeated cycles of reproduction, if occurring within the stress “window,” would select in favor of recombinants with new combinations of heat tolerance genes, expressing stacked tolerance not observed before in the population. Thus new genotypes will have increased tolerance to the same specific stress that originally caused male sterility in their maternal line. Heat extremes thus generate progeny with increased heat tolerance. This is the crux of the Diversity Assurance concept we propose, which goes beyond reproduction assurance.

If stress caused female sterility before male sterility, this would not enhance effective outcrossing. Thus it makes evolutionary sense that male sterility under extreme stress is indeed more common, because recombinant offspring carry both the male-sterility-under-stress trait of the female parent and the specific stress tolerance from both parents. As stress tolerance is thus enhanced in the progeny, the continued presence of the proposed male-sterility-under-stress mechanism allows it to enhance outcrossing once again when external stresses further escalate.

## 8. Conclusions

Diversity Assurance represents a decisive advance over mere reproductive assurance because it enables recombination, evolution and adaptation in the presence of severe stress in specific highly self-fertilizing plants. We have focused on heat stress, but the same reasoning can be made for differential male sterility caused by other stresses, with pollen formation even described as the Achilles tendon of plant reproduction [68]. Most crucially, this proposed mechanism also contributes in part to explaining the very persistence of self-fertilizing species in nature. Male-sterility-facilitated Diversity Assurance thus offers a challenge to Stebbins’ argument that selfing will lead to an evolutionary dead end. Darwin, in 1859, wrote: “... no organic being self-fertilises itself for an eternity of generations; but that a cross with another individual is occasionally – perhaps at very long intervals – indispensable. ... in the long run, the influence of intercrosses, even at rare intervals, will be great.” [69]

Rather than just as an unfortunate debilitating oddity, stress-induced male sterility can in specific circumstances act as a constructive, enabling factor in the struggle for existence. Such reproductive

adaptations are expected to increase under further climate change [2]. As indicated, hard data on the issues involved in wild populations are very rare, and therefore we suggest that wild populations be studied for the presence of this proposed mechanism under controlled field studies in their native habitats.

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