

CAN GREENLIP (*HALIOTIS LAEVIGATA*) ABALONE BREEDING PROGRAMS TOLERATE FLUCTUATIONS IN REPRODUCTIVE PERFORMANCE?

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ABSTRACT Fluctuations in reproductive performance (i.e., spawning success, hatching rate, larval survival) are a common occurrence in abalone breeding programs, in particular during the early stages of their development. Such fluctuations affect the numbers of families available for progeny testing and selection, and can have consequences for genetic gains and inbreeding. We used stochastic computer simulations to understand how genetic gains and levels of inbreeding are affected when greenlip (*Haliotis laevigata*) breeding programs encounter varying severity and frequency of reproductive failure. We simulated breeding programs for greenlip abalone with both conservative and aggressive selection approaches over 35 y (10 generations). Without reproductive failure, genetic improvements of 36%–55% could be achieved after 10 y of selection in a single trait in a commercial abalone breeding program with a conservative selection approach, and gains of twice that could be achieved with a selection approach that allowed high rates of inbreeding. A conservative selection approach would be sustainable even at high rates of reproductive failure, whereas a more aggressive approach would lead to nearly twice the recommended level of inbreeding. It was concluded that breeding programs for greenlip abalone may be buffered against unexpected fluctuations in reproductive performance if the selection approach is chosen strategically.

KEY WORDS: abalone, selective breeding, genetic gain, inbreeding, simulation, *Haliotis laevigata*

INTRODUCTION

Reproductive success rates less than 100% are a challenge for captive abalone breeding programs (Lemay & Boulding 2009) and can be expected to be as low as 40% in a commercial setting (Elliott 2000). Causes for low rates of reproductive success are complex and can occur at various stages of the reproductive cascade, from spawning to juvenile survival. Among others, they can include factors that influence spawning (Grubert et al. 2005, Nuurai et al. 2010), a lack of synchronicity of spawning in males and females (Grubert et al. 2005), factors that influence fertilization rates (Encena et al. 1998, Babcock & Keesing 1999, Huchette et al. 2004, Grubert et al. 2005, Suphamungmee et al. 2010), or factors that influence postlarval survival (Takami et al. 2002, Lucas et al. 2006, Symonds et al. 2012). The consequence of decreased reproductive success is a lower number of surviving families, which limits the number of source families when selecting parents for the next generation. In a broad-spawning species like abalone, this problem can even be enhanced by unequal contributions of individual parents (Evans et al. 2004a, Hara & Sekino 2007, Symonds et al. 2012).

In a breeding program of specific size, as defined by the number of families, sufficient parents need to be selected every year to produce adequate numbers of families to maintain the size of the breeding program. If not all families are available for selection, more individuals from the same families need to be selected and would therefore have a higher degree of relatedness. This can have a positive effect on genetic gains because more individuals of the best-performing families are selected; however, levels of inbreeding may also increase as a consequence. This

trend is applicable to any breeding program that encounters limitations of available selection candidates.

The main objective in selective breeding programs is to balance genetic gains and inbreeding. Increased genetic gains are achieved by selecting outstanding, but often related, individuals. Such a practice increases the rate of inbreeding, which might reduce variation and fitness and affect long-term sustainability of the breeding program. These effects, in combination with decreased production, have been documented in livestock, particularly for reproductive and fitness characteristics (Falconer & Mackay 1989). Publications on effects of inbreeding depression on production traits in abalone species and other shellfish species are scarce. In Pacific abalone, inbreeding has been reported to affect deformities in veliger larvae and survival after settlement (Park et al. 2006, Kobayashi & Kijima 2010), and metamorphic success (Deng et al. 2005). Evans et al. (2004b) reported inbreeding effects in Pacific oysters, even among crosses of distantly related parents, that affected yield, individual growth rate, and survival. Rates of inbreeding per generation of 1% have been recommended to balance inbreeding effects in aquaculture mass selection programs (Frankel & Soulé 1981, Bentsen & Olesen 2002).

Stochastic computer simulation is often used to investigate the effect of selection strategies on genetic gain and inbreeding in aquaculture breeding programs (Hayes et al. 2007, Nielsen et al. 2011, Hely et al. 2012). The advantage of stochastic simulation is that it can mimic complex processes in a whole population realistically by simulating each individual (Dekkers et al. 2004). The biggest disadvantage is that it is computationally demanding and therefore time-consuming (Dekkers et al. 2004).

Using stochastic simulation, this study estimated the genetic gains that may be achieved in commercial greenlip (*Haliotis laevigata*) abalone breeding programs at 2 predetermined levels

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of inbreeding. In a second step, varying frequencies and severities of reproductive failure were imposed to investigate the effects of a limited number of families on genetic gains and the rate of inbreeding.

MATERIALS AND METHODS

Simulation of the Base Breeding Program

Stochastic computer simulation was used to explore different breeding program scenarios for greenlip abalone. Initially, a base breeding program was simulated to investigate potential genetic gains and levels of inbreeding that can be achieved in a commercial greenlip abalone breeding program. For this purpose, the simulation implemented mating structures and generation intervals that are characteristic to a commercial greenlip abalone breeding program. The base breeding program assumed single abalone species improvement in a single production trait. Genetic parameter estimates for production traits in abalone are rare and vary widely. Low heritabilities were estimated in greenlip abalone ($h^2 = 0.01\text{--}0.16$) for shell length, total weight, and meat weight (Kube et al. 2007); moderate to high heritabilities of $h^2 = 0.36\text{--}0.48$ were estimated for weight, shell length, and width in tropical abalone (Lucas et al. 2006); and $h^2 = 0.40\text{--}0.55$ in New Zealand abalone for shell length and width (Symonds et al. 2012). Therefore, a conservative heritability (h^2) of 0.3 was chosen to represent the ratio of genetic and phenotypic variance of the production trait under selection. The mean of the trait (μ) was assumed to be 5, without a specific unit, and the environmental mean was 0. A phenotypic and environmental variance of 1 and 0.7, respectively, were assumed, and the resulting coefficient of variation was 0.2.

The simulation commenced with an unrelated base population of greenlip abalone, assuming that the founder stock originated from different populations, and the selective breeding program was run over 35 y, or 10 generations. For each founder individual, a phenotype was simulated as the sum of a simulated genotype and environmental effect, using the described mean and variances. Every year, superior males and females were selected, and each male and female mated twice (2×2 mating structure). Individuals are bred with the genotype for progeny simulated as the mean of the parents' genotypes plus a random Mendelian sampling term drawn from a normal distribution with 0 mean. If the parents are not inbred, the variance of the Mendelian sampling term is one half of the genetic variance. If the parents are inbred, then the variance of the Mendelian sampling term is less than one half of the genetic variance. Pedigree information and mate allocation were used to avoid mating of closely related individuals. Selection was based on estimated breeding values, which were generated using

ASReml (Gilmour et al. 2009). All individuals were only used once as parents and there were no genetic links between years. The generation interval was 3 y for males and 4 y for females, yielding an average generation interval of 3.5 y. Communal rearing effects, which may be present in abalone family rearing systems, were not simulated, assuming in practical terms that families are mixed at tagging. It was assumed that performance records for the trait under selection were recorded on 100 progeny per family. Full pedigree was kept, so that the coefficient of inbreeding was known in relation to the unrelated base population. The random element of stochastic simulation generates variation in the results. Therefore, each breeding program scenario was simulated 25 times, and the mean results for genetic improvement and inbreeding were reported.

Simulation of Fluctuation in Reproductive Performance

For the purpose of this study, good reproductive performance was defined as the successful mating of a male and female individual resulting in a new family. The effect of fluctuating reproductive performance was simulated by imposing different levels of severity of reproductive failure and different frequencies of occurrence of reproductive failure on the base breeding program. Parameters for the base breeding program, such as variances and mating structure, were kept constant for all scenarios. Table 1 summarizes the different frequencies and levels of severity of reproductive failure that were simulated. As an additional variable, the effect of the size of the breeding program, defined by the numbers of families in the breeding operation, was investigated. Breeding programs with 24, 48, or 96 families per year were simulated to study the interaction between fluctuating reproductive performance and size of the breeding program. All combinations of varying sizes of breeding programs, levels of severity, and frequency of reproductive failure using 2 selection approaches that are described in the following were simulated, resulting in 78 different simulation scenarios. Not all results are reported.

Simulation of Different Selection Approaches

The attitude to inbreeding in a breeding program defines the level of relatedness tolerated in the selection candidates and determines the maximum genetic gains that can be achieved. Two selection approaches that characterize a breeding operation's attitude to inbreeding were simulated to investigate the consequences of reproductive failure on genetic gains and inbreeding. The aggressive selection approach is characterized by a higher acceptance of risk to inbreeding, and placed emphasis on the selection of genetically superior individuals, which are often closely related. This selection approach was

TABLE 1.
Overview of parameters that define the breeding program scenarios.

| Parameter | 24 | 48 | 96 | Never |
|--|------------------------------------|----------|--------------------------------------|-------|
| No. of families per year | 24 | 48 | 96 | |
| Frequency of reproductive failure | 1 in 1 y | 1 in 2 y | 1 in 3 y | Never |
| Severity of reproductive failure | 8.3% | 25% | 41.7% | 83% |
| Selection approach (base breeding program) | Aggressive mean $\Delta F = 1.5\%$ | | Conservative mean $\Delta F = 0.6\%$ | |

predetermined so that the average rate of inbreeding ranged from $\Delta F = 1.45\%$ – 1.75% per generation, which exceeded the recommended inbreeding of a long-term, sustainable breeding program of $\Delta F = 1\%$ (Frankel & Soulé 1981). It represents an option that, in the medium to long term, would require the introduction of unrelated genetic stock to mitigate consequences of inbreeding. The low-risk selection approach to inbreeding, referred to as the conservative approach, could represent a breeding program for a closed or small population. The mean rate of inbreeding per generation ranged from $\Delta F = 0.45\%$ – 0.75% , which results from a deliberate emphasis on a low level of relatedness in the selection candidates.

In the simulation, the mean inbreeding levels of the 2 selection approaches were managed by controlling the maximum grandparental contribution to the offspring generation. For example, a grandparental contribution of 4 means that each grandsire and granddam can be represented up to 4 times via the selected parents. The number of times grandparents should contribute to achieve the specified levels of mean inbreeding for the aggressive and conservative selection approaches was established prior to the simulation runs. Table 2 shows the numbers for the genetic contribution of grandparents, dependent on breeding operation size, and the resulting rate of inbreeding. The genetic contributions shown in Table 2 served as input parameters for the simulation of the main study.

If the constraints of relatedness of the selection approach could not be met, for example if there was a lack of unrelated selection candidates resulting from reproductive failure, the constraints were relaxed and higher grandparental genetic contributions were allowed. The simulation handles the relaxation of the inbreeding constraints logically, but somewhat opportunistically, simulating a real-world approach that a breeding program manager might take if sophisticated decision-making tools on how to apply the relaxation were not available. If constraints for grandparental contributions cannot be met, because of a lack of unrelated families, there will be some individuals that need to be selected from related families, which increases the grandparental contribution. The software generates different options, selecting these individuals from different related families. Each additional individual that exceeds the grandparental contribution threshold gets scored as a penalty. Different options can generate the same penalty score, but vary in the resulting level of relatedness in the selection candidates. The decision on how the selection of these individuals is distributed across different related families is based on the penalty score and

the genetic superiority of the selected individuals. The simulation uses the option with the lowest penalty score and the highest genetic superiority. The level of relatedness is still considered, but the applied option is opportunistic in favor of the achieved genetic gains.

RESULTS

Genetic Gain Without Reproductive Failure

The simulations of abalone breeding programs without reproductive failure aimed for constant rates of inbreeding by varying the grandparental genetic contributions, depending on the size of the breeding operation (Table 3). In breeding operations with 24 or 48 families, a conservative selection approach resulted in rates of inbreeding of $\Delta F = 0.49\%$ and $\Delta F = 0.45\%$, respectively, and a higher rate of inbreeding at $\Delta F = 0.73\%$ with 96 families. With an aggressive selection approach, breeding programs achieved an inbreeding rate of $\Delta F = 1.64\%$ with 24 and 48 families, and $\Delta F = 1.48\%$ with 96 families.

With successful reproduction, genetic improvement over 10 generations was twice as high for breeding programs with an aggressive selection approach (78%–120%, Table 3) compared with a conservative approach with the same number of families (36%–55%, Table 3).

Genetic improvement increased with increasing number of families in the breeding program, but the increase was lower for a conservative selection approach compared with an aggressive one. With a conservative selection approach, 19% more gain was achieved by increasing family numbers from 24–96 families. However, with an aggressive selection approach, the gain increased by 42% with the same increase in family numbers.

Effect of Size of Breeding Population with Reproductive Failure

The effect of reproductive failure on breeding programs of different size was explored by simulating a constant occurrence of reproductive failure of 1 in every 3 y, affecting 41.7% of families (Table 3). Therefore, triennial reproductive failure affecting 41.7% of families meant that breeding programs with 24 families would only have 14 families available for selection in the next generation, whereas programs of 48 families were reduced to 28 families, and breeding programs of 96 families were reduced to 56 families.

The rate of inbreeding and genetic gains increased when reproductive failure occurred. Mean ΔF increased to 0.75% and 1.86% under conservative and aggressive selection approaches, respectively. Meanwhile, genetic gain increased by approximately 24% and 37% for the conservative and aggressive selection approaches, respectively, with increasing size of the breeding program.

Effect of the Frequency of Reproductive Failure

Genetic improvement and rate of inbreeding increased with increasing frequency of reproductive failure (Table 4). Relative to the base breeding programs, annual reproductive failure increased genetic improvement by 17% and ΔF doubled under a conservative selection approach.

The effect of the frequency of reproductive failure was higher in breeding programs with an aggressive selection approach.

TABLE 2.

Genetic contribution (GenCont) of grandparents to achieve a specified rate of inbreeding per generation ranging from $\Delta F = 1.45 - 1.75\%$ (aggressive selection approach) and $\Delta F = 0.45 - 0.75\%$ (conservative selection approach) in breeding operations of 3 sizes.

| Selection Approach | Size of Breeding Operation | | | | | |
|--------------------|----------------------------|----------------|-------------|----------------|-------------|----------------|
| | 24 Families | | 48 Families | | 96 Families | |
| | GenCont | ΔF (%) | GenCont | ΔF (%) | GenCont | ΔF (%) |
| Conservative | 2 | 0.49 | 4 | 0.45 | 8 | 0.73 |
| Aggressive | 5 | 1.64 | 8 | 1.64 | 20 | 1.48 |

TABLE 3.

Total genetic improvement and rate of inbreeding per generation (ΔF) after 10 generations of selection without reproductive failure and with triennial reproductive failure affecting 41.7% of families.

| No. of Families | Selection Approach | Genetic Improvement without Failure (%) | ΔF without Failure (%) | Genetic Improvement with Triennial Failure (%) | ΔF with Triennial Failure (%) |
|-----------------|--------------------|---|--------------------------------|--|---------------------------------------|
| 24 | Conservative | 35.81 | 0.49 | 37.80 | 0.64 |
| 48 | | 38.87 | 0.45 | 47.44 | 0.64 |
| 96 | | 54.96 | 0.73 | 62.15 | 0.97 |
| 24 | Aggressive | 78.03 | 1.64 | 89.23 | 1.95 |
| 48 | | 85.83 | 1.64 | 93.13 | 1.82 |
| 96 | | 120.12 | 1.48 | 126.66 | 1.81 |

Data are shown for 2 selection approaches and for different-size breeding populations (no. of families).

Genetic improvement increased by 24% and ΔF increased to 2.22%.

Effect of Severity of Reproductive Failure

Genetic improvement and the rate of inbreeding increased with increasing number of families affected by reproductive failure (Table 5). For conservative selection strategies in which 41.7% of families were affected by reproductive failure, the increase in the rate of inbreeding was only 0.19%. However, severe failure affecting 83% of families increased ΔF to 1.53%, which is above the recommended maximum rate of ΔF of 1%. The same trend could be observed in genetic improvement, with a relatively small increase of 10% at a rate of failure of 41.7% versus an increase of 42% with maximum simulated reproductive failure.

The effects on genetic improvement and the rate of inbreeding were less for breeding programs with an aggressive selection approach compared with a conservative approach. Compared with no reproductive failure, the rate of inbreeding increased by only 0.25% and genetic improvement by 7.5% with 83% of families affected.

DISCUSSION

Commercial greenlip abalone breeding programs can achieve, on average, between 36% and 120% genetic improvement in a single trait over 35 y of selection, depending on the size of the breeding operation and the tolerated rate of inbreeding per generation. These rates of genetic gain over the specified time period are specific to the greenlip abalone simulation scenarios and are dependent on the heritability of the trait, the generation interval, and the superiority of the selected individuals. For example, genetic gains would be higher if the trait under selection has a higher heritability; genetic gains would be lower over the 35-y time frame if an abalone species with a longer generation interval is considered (Falconer & Mackay 1989).

Without reproductive failure in the breeding programs, 2 main trends were observed in this simulation study. First, genetic gain increased with increasing numbers of families at a constant rate of inbreeding. The constant rate of inbreeding was determined by the grandparental contributions to the next generation, and restricted the number of related individuals that could be selected from each family. In larger breeding operations, even though more individuals have to be selected as

parents relative to the total number of families, fewer unrelated families need to be selected at a constant rate of inbreeding. For example, at a constant rate of inbreeding ($\Delta F = 1.64\%$), 5 grandparental contributions to the next generation are allowed in a breeding operation with 24 families, whereas this number increases to 8 with 48 families (Table 2), which means that fewer unrelated families have to be selected. The availability of more families offers more potential to select high-performing individuals across families and therefore increases genetic gains. However, in reality, the greater availability of families might still not safeguard from unequal contributions of particular individuals and yield the anticipated genetic gain because the number of actual broodstock that successfully reproduce can be low (Evans et al. 2004a, Hara & Sekino 2007).

The second trend observed was that an increase in the tolerated rate of inbreeding per generation increased genetic improvement. The more aggressive the selection approach, the more related individuals could be selected. Therefore, the overall selection intensity could be increased because more genetically superior individuals from the best families could be selected. Also, fewer families have to be selected, which is similar to the effect of having more families in the breeding operation. These trends for the effect of the size of the breeding operation on genetic improvement and inbreeding were also apparent when reproductive failure was imposed on the breeding program.

TABLE 4.

Total genetic improvement and rate of inbreeding per generation (ΔF) after 10 generations of selection with varying frequencies of reproductive failure affecting 41.7% of families, 2 selection approaches, and a breeding population of 48 families.

| Frequency of Failure | Selection Approach | Genetic Improvement (%) | ΔF (%) |
|----------------------|--------------------|-------------------------|----------------|
| No failure | Conservative | 38.88 | 0.45 |
| 1 in 3 y | | 47.44 | 0.64 |
| 1 in 2 y | | 49.21 | 0.68 |
| Every year | Aggressive | 55.65 | 0.96 |
| No failure | | 85.83 | 1.65 |
| 1 in 3 y | | 93.13 | 1.82 |
| 1 in 2 y | | 99.97 | 1.89 |
| Every year | | 110.45 | 2.22 |

TABLE 5.
Total genetic improvement and rate of inbreeding per generation (ΔF) after 10 generations of selection with varying severity of triennial reproductive failure, 2 selection approaches, and a breeding population of 48 families.

| Severity of Failure (% tanks failed) | Selection Approach | Genetic Improvement (%) | ΔF (%) |
|---|--------------------|----------------------------|----------------|
| No failure | Conservative | 38.87 | 0.45 |
| 8.3 | | 40.59 | 0.48 |
| 25 | | 43.06 | 0.53 |
| 41.7 | | 47.44 | 0.64 |
| 83 | | 80.76 | 1.53 |
| No failure | Aggressive | 85.83 | 1.65 |
| 8.3 | | 85.96 | 1.69 |
| 25 | | 86.75 | 1.78 |
| 41.7 | | 93.13 | 1.82 |
| 83 | | 93.27 | 1.94 |

Increasing the size of the breeding operation had a less pronounced effect on the increase in genetic gain compared with tolerating high rates of inbreeding and running a more aggressive selection approach. The reason is that increasing the size of the breeding operation only increases the number of families from which to select, but the constraint on related animals remains and restricts the numbers of superior individuals that can be selected within families. However, it is unlikely that tolerating a high level of inbreeding is a sustainable approach to increase genetic gains in the long term. Ultimately, it will become necessary to introduce unrelated stock to manage inbreeding. This point was also emphasized by Brown et al. (2005) in relation to commercial breeding programs of gilthead seabream. For a closed breeding operation, an increase in family numbers and, therefore, potential individuals to contribute to the next generation enables an increase in genetic gain with the ability to maintain inbreeding at low levels.

This study simulated the effect of reproductive failure in abalone breeding operations with whole families failing and, as a consequence, limited numbers of families being available for selection of parents for the next generation. This affects the genetic gains that can be achieved and it can also pose a challenge to maintain sustainable levels of inbreeding in the breeding operation. Two factors—frequency and the severity of reproductive failure—were investigated for their effect on genetic gains and rate of inbreeding. With increasing severity and frequency of failure, both the genetic improvement and the rate of inbreeding increased. This result was counterintuitive because we established that more families in a breeding operation result in larger genetic gains. In this case, reproductive failure resulted in fewer families, and the expectation would be for genetic gains to decrease. However, the decrease in family numbers meant that the constraints on relatedness in the selection candidates could not be met. To select sufficient individuals as parents for the next generation, the constraints on the relatedness of selection candidates had to be relaxed and more related individuals needed to be selected. The algorithm that determined the families with increased grandparental contributions put some emphasis on minimizing relatedness, but tried to maximize genetic gain at the same time. It simulated the likely decision-making process faced by a breeding program

manager forced to select more related individuals than planned, where more of the better performing families are likely to be selected. As a consequence, there is a positive effect on the genetic gain at an increased rate of inbreeding with decreasing numbers of families. The relaxation of constraints was applied to make the simulation more realistic and, as a consequence these results, are initially surprising, but are in accordance with the expectations of the equations of Falconer and Mackay (1989) for response to selection.

At low to medium frequencies of reproductive failure or an occurrence of failure once every 2–3 y, effects on inbreeding and genetic gain were low, but an increase could be observed. The reason is that some accumulation of relatedness in the later years of the breeding program occurred, forcing the simulation to relax the constraints on the selection of related animals. Therefore, the reported mean genetic improvement over 35 y was increased compared with breeding programs that did not encounter reproductive failure. Failure was simulated to only occur once every 3 y; therefore, low to medium failure of 8.3% and 25% of families, respectively, did not have a strong effect. In the earlier years of the breeding program, the constraints that were imposed on the rate of inbreeding under both the conservative and aggressive selection approach could be met, even with reduced number of families. Nevertheless, an increased level of inbreeding indicates that these could not be met in the later years of the breeding program after relatedness started to accumulate.

Effects of frequency and severity of reproductive failure on genetic improvement and level of inbreeding were similar for aggressive and conservation selection approaches. However, tolerance of an already high level of inbreeding affects the sustainability of the breeding program. A reduction in numbers of families resulting from reproductive failure quickly pushes the relatedness in the population to levels that are inadvisable, even in the short term. With an annual failure of 41.7% of families, the rate of inbreeding for the conservative approach still did not exceed the recommended threshold of 1% (Frankel & Soulé 1981). However, for the aggressive approach, annual failure of 41.7% of families increased the rate of inbreeding to more than twice the recommended 1% per generation (Frankel & Soulé 1981). For the same reasons as outlined earlier, the effects on genetic improvement were positive.

The trends observed in this simulation study can also be related to family-based selective breeding programs in other aquaculture species, because the heritability used in this study is generally valid for production traits across various species (Kanchanachai et al. 2011, Bentsen et al. 2012, Sae-Lim et al. 2013). However, assumptions on the generation interval and mating structure were characteristic to breeding programs for greenlip abalone and influence genetic gains over the time frame that was used in this study. This is the first study that investigated specifically the effect of reproductive failure in an aquaculture species in a simulation study. Other work looking at genetic diversity based on genetic markers in different abalone species (Evans et al. 2004a, Hara & Sekino 2007, Van den Bergb & Roodt-Wilding 2010), gilthead seabream (Brown et al. 2005), and Pacific oysters (Boudry et al. 2002) support the results of this study from a molecular perspective. Sufficient parents are required to ensure long-term genetic diversity, which is particularly important in a closed breeding program.

In conclusion, the results demonstrate that abalone breeding programs with a conservative attitude to inbreeding may be sustainable in the long term, even with a low to moderate occurrence of reproductive failure. Breeding programs that

tolerate higher rates of inbreeding are pushed to undesirable levels of inbreeding with reproductive failure. Even without reproductive fluctuations, aggressive selection approaches are only sustainable in the long term if unrelated stock can be introduced.

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