Conservation Genetics Module for *Pycnopodia helianthoides*: First-Principles Framework for Breeding Program Design

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Abstract

This document develops the theoretical framework for using the SSWD-EvoEpi agent-based model to inform conservation breeding and reintroduction of *Pycnopodia helianthoides* (sunflower sea star). We derive from first principles the quantitative genetics of polygenic disease resistance, the statistical theory of founder screening, optimal breeding program design under inbreeding constraints, and the population genetics of genetic rescue through reintroduction. Each section connects mathematical theory to the specific genetic architecture implemented in the model (51 biallelic loci across three traits), generating concrete predictions that become testable once the model is calibrated. The module includes code implementations of all theoretical results and analysis templates ready for execution with final model parameters.

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1 Introduction

Sea star wasting disease (SSWD) has caused one of the largest documented mass mortality events in a marine invertebrate, reducing *Pycnopodia helianthoides* populations by an estimated 90–99% across its range from Alaska to Baja California [Harvell et al., 2019, Montecino-Latorre et al., 2020]. As a keystone predator of urchins, the loss of *Pycnopodia helianthoides* has triggered trophic cascades leading to kelp forest collapse in multiple regions [Schultz et al., 2016].

Conservation efforts now center on captive breeding and reintroduction [Sea Star Lab, Friday Harbor Laboratories, 2024]. The first caged outplanting trials were conducted at Friday Harbor Laboratories in 2023, followed by the first open release of 20 captive-bred juveniles in July 2024, and a California release of 47 juveniles in December 2025 (with 46/47 surviving the first month). These programs face a fundamental question: **how do we design a breeding program that maximizes disease resistance in released stock while maintaining sufficient genetic diversity for long-term population viability?**

1.1 What this module provides

The SSWD-EvoEpi model tracks individual genotypes at 51 biallelic loci controlling three disease-related traits (resistance, tolerance, recovery). This individual-level genetic resolution enables analyses that population-level models cannot:

1. **Predicted genetic state.** What do the trait distributions of surviving wild populations look like at each site, right now?
2. **Screening effort.** How many wild individuals must be sampled to find founders with desired resistance levels?
3. **Breeding optimization.** Which crossing strategy (random, assortative, complementary) maximizes resistance gain per generation, and at what cost to genetic diversity?
4. **Reintroduction design.** How many captive-bred individuals, released where and when, shift the evolutionary trajectory of wild populations?

1.2 Approach

Each section of this document follows the same structure:

- **First-principles derivation** of the relevant quantitative genetics or population genetics theory
- **Mapping to our model** — how the general theory specializes to 51 loci, 3 traits, and biallelic architecture
- **Computable predictions** — equations that take model parameters as input and produce conservation-relevant output
- **Implementation notes** — pointers to the code that implements each result

All derivations assume the calibrated model is available. Until then, we use validation-run parameters as placeholders and note where results will change.

1.3 Scope and limitations

This module addresses the *genetics* of conservation. It does not address habitat restoration, water quality management, or disease treatment — only the question of which individuals to breed, how to cross them, and where to release their offspring. The model does not currently include inbreeding depression (reduced fitness from homozygosity of deleterious recessives), which we flag as a known gap throughout.

2 Genetic Architecture

Before deriving population-level predictions, we must formalize the individual-level genetics. Everything downstream depends on getting this right.

2.1 Locus structure

The model implements 51 biallelic loci, motivated by the Schiebelhut et al. [2018] genome scan which identified 51 loci showing significant allele frequency shifts between pre- and post-SSWD *Pycnopodia helianthoides* populations. These are partitioned into three non-overlapping trait blocks:

$$n_R = 17 \quad \text{(resistance loci, indices 0–16)} \tag{1}$$
$$n_T = 17 \quad \text{(tolerance loci, indices 17–33)} \tag{2}$$
$$n_C = 17 \quad \text{(recovery loci, indices 34–50)} \tag{3}$$

The partition is configurable (`n_resistance`, `n_tolerance`, `n_recovery` in model config), subject to the constraint $n_R + n_T + n_C = 51$.

2.2 Genotype representation

Each individual i carries a diploid genotype at each locus ℓ:

$$g_{i,\ell} = (a_{i,\ell,1},\, a_{i,\ell,2}) \in \{0,1\}^2 \tag{4}$$

where $a = 1$ denotes the derived (protective) allele and $a = 0$ the ancestral allele. The genotypic value at locus ℓ is the allele mean:

$$x_{i,\ell} = \frac{a_{i,\ell,1} + a_{i,\ell,2}}{2} \in \{0,\, 0.5,\, 1\} \tag{5}$$

2.3 Effect sizes

Each trait block has effect sizes α_ℓ drawn from $\text{Exp}(\lambda)$ and normalized to sum to 1:

$$\alpha_\ell^{(\text{raw})} \sim \text{Exp}(1), \qquad \alpha_\ell = \frac{\alpha_\ell^{(\text{raw})}}{\sum_{j=1}^{n} \alpha_j^{(\text{raw})}} \tag{6}$$

so $\sum_{\ell=1}^{n} \alpha_\ell = 1$ exactly. Effect sizes are sorted descending: $\alpha_1 \geq \alpha_2 \geq \cdots \geq \alpha_n$.

This creates a realistic effect-size distribution where a few loci contribute disproportionately — consistent with the observation that complex trait architectures typically show a roughly exponential distribution of effect sizes [Barton and Keightley, 2002].

Remark 2.1. *Because $\sum \alpha_\ell = 1$, the trait value of a fully homozygous-derived individual ($x_{i,\ell} = 1$ at all loci) is exactly* 1.0*. This makes trait values interpretable as a fraction of the theoretical maximum.*

2.4 Trait computation

The trait score for individual i in trait block $\mathcal{L}$ (with effect sizes $\boldsymbol{\alpha}$) is:

$$\tau_i = \sum_{\ell \in \mathcal{L}} \alpha_\ell \, x_{i,\ell} = \boldsymbol{\alpha}^T \mathbf{x}_i \tag{7}$$

This is a **strictly additive** model: no dominance, no epistasis. The trait value is the weighted sum of allelic dosages. This means:

- Trait values are continuous on $[0, 1]$
- The distribution is determined entirely by allele frequencies and effect sizes
- Heterozygotes are exactly intermediate (no dominance deviation)
- Traits from different blocks are genetically independent (no pleiotropy, no linkage)

2.5 Three traits and their phenotypic effects

Each trait controls exactly one aspect of the host–pathogen interaction:

2.5.1 Resistance (r_i)

Immune exclusion. Reduces the instantaneous infection hazard rate:

$$\lambda_i = a \cdot \frac{P_k}{K_{1/2} + P_k} \cdot (1 - r_i) \cdot S_{\text{sal}} \cdot f_{\text{size}}(L_i) \tag{8}$$

The daily infection probability is $p_{\text{inf}} = 1 - e^{-\lambda_i}$. At $r_i = 1$, $\lambda_i = 0$: complete immunity. At $r_i = 0$, full susceptibility.

Note: Resistance is multiplicative in the hazard rate. This means the fitness benefit of each unit increase in r depends on pathogen pressure. In low-pathogen environments, the marginal value of resistance is small. In high-pathogen environments, resistance is strongly selected.

2.5.2 Tolerance (t_i)

Damage limitation. Extends survival time in the I_2 (symptomatic terminal) stage by reducing the $I_2 \to D$ transition rate:

$$\mu_{I_2D}^{\text{eff}} = \mu_{I_2D} \cdot (1 - t_i \cdot \tau_{\max}), \qquad \text{floor: } \mu_{I_2D}^{\text{eff}} \geq 0.05 \cdot \mu_{I_2D} \tag{9}$$

where $\tau_{\max} = 0.85$. At maximum tolerance ($t_i = 1$), the I_2 stage is extended by a factor of $1/(1 - \tau_{\max}) = 6.67$. The 5% floor prevents immortality.

2.5.3 Recovery (c_i)

Pathogen clearance. Daily probability of recovery from I_2:

$$p_{\text{rec}} = \rho_{\text{rec}} \cdot c_i \tag{10}$$

where $\rho_{\text{rec}} = 0.05$ d^{-1}. Additionally, early recovery from I_1 is possible if $c_i > 0.5$:

$$p_{\text{early}} = \rho_{\text{rec}} \cdot 2 \cdot (c_i - 0.5), \qquad c_i > 0.5 \tag{11}$$

2.6 Allele frequency initialization

Per-locus protective allele frequencies are drawn from a scaled Beta distribution:

$$q_\ell^{(\text{raw})} \sim \text{Beta}(a, b), \qquad q_\ell = \text{clip}\left(q_\ell^{(\text{raw})} \cdot \frac{\bar{\tau}_{\text{target}}}{\boldsymbol{\alpha}^T \mathbf{q}^{(\text{raw})}},\ 0.001,\ 0.5 \right) \tag{12}$$

with $a = 2$, $b = 8$ (right-skewed, most loci at low frequency). The scaling ensures $\mathbb{E}[\tau_i] \approx \bar{\tau}_{\text{target}}$.

The clip to $[0.001, 0.5]$ is important: it ensures no locus is fixed (always some variation to select on) and no locus has the protective allele at majority frequency (consistent with the assumption that SSWD-protective alleles were rare pre-epidemic).

2.7 Key properties for breeding

Proposition 2.1 (Trait independence)**.** *Because the three trait blocks occupy non-overlapping loci, and allele frequencies are drawn independently per block, the trait values (r_i, t_i, c_i) are statistically independent at the population level. The correlation between any two traits is $\rho \approx 0$ (confirmed empirically: $|\rho| < 0.005$ in simulations with $N = 100{,}000$).*

Proposition 2.2 (Maximum possible trait value)**.** *An individual homozygous for the protective allele at all n loci of a trait block achieves $\tau_i = \sum_\ell \alpha_\ell \cdot 1 = 1.0$. The probability of this occurring in a random individual is:*

$$\mathbb{P}(\tau_i = 1) = \prod_{\ell=1}^{n} q_\ell^2 \tag{13}$$

which is astronomically small for typical q_ℓ values (~ 0.1–0.3).

Proposition 2.3 (Additive variance)**.** *For a biallelic locus with frequency q and effect α, the additive genetic variance contribution is:*

$$V_{A,\ell} = 2q_\ell(1 - q_\ell)\alpha_\ell^2 \tag{14}$$

The total additive variance for a trait is:

$$V_A = \sum_{\ell=1}^{n} 2q_\ell(1 - q_\ell)\alpha_\ell^2 \tag{15}$$

This is exact under our purely additive model (no dominance or epistatic variance).

3 Quantitative Genetics of Selection Response

Given the genetic architecture defined in Section 2, we now derive the expected response to selection — the central quantity for breeding program design.

3.1 The breeder's equation

The fundamental prediction tool in quantitative genetics is the breeder's equation [Lush, 1937, Falconer and Mackay, 1996]:

$$R = h^2 \cdot S \tag{16}$$

where:

- $R = \bar{\tau}_{t+1} - \bar{\tau}_t$ is the response to selection (change in population mean trait value per generation)
- $h^2 = V_A/V_P$ is the narrow-sense heritability
- $S = \bar{\tau}_{\text{selected}} - \bar{\tau}_t$ is the selection differential (difference between the mean of selected parents and the population mean)

3.2 Heritability in our model

In the SSWD-EvoEpi model, traits are purely genetically determined with no environmental variance:

$$V_P = V_A + \underbrace{V_D}_{=0} + \underbrace{V_E}_{=0} = V_A \tag{17}$$

Under strict additivity (no dominance), $V_D = 0$. We impose no environmental stochasticity on trait expression, so $V_E = 0$. Therefore:

$$h^2 = 1.0 \quad \text{(in the model)} \tag{18}$$

Remark 3.1. *This is an idealization. In real Pycnopodia helianthoides, heritability of disease resistance is unknown but certainly* < 1*. Environmental factors (nutritional state, temperature stress, prior infections, microbiome composition) would add* $V_E > 0$*, reducing* h^2*. However, for the purpose of* relative *comparisons between breeding strategies within the model,* $h^2 = 1$ *simplifies the analysis without affecting ranking of strategies. When interpreting absolute generation counts, we should apply a correction factor* $h^2_{real}/1.0$ *to account for this.*

3.3 Selection differential from truncation selection

In a breeding program, truncation selection retains the top fraction p of individuals. The selection differential is:

$$S = i \cdot \sigma_P \tag{19}$$

where i is the **selection intensity**, a function of p alone.

For a normal distribution (approximately valid for polygenic traits with many loci):

$$i = \frac{\phi(z_p)}{1 - \Phi(z_p)} = \frac{\phi(z_p)}{p} \tag{20}$$

where Φ is the standard normal CDF, ϕ is the PDF, and $z_p = \Phi^{-1}(1-p)$ is the truncation point.

Fraction selected (p)	Selection intensity (i)	Practical meaning
0.50	0.80	Keep top half
0.20	1.40	Keep top fifth
0.10	1.76	Keep top tenth
0.05	2.06	Intensive selection
0.01	2.67	Extreme selection

3.4 Predicted gain per generation

Combining Equations (16) and (19) with $h^2 = 1$:

$$R = i \cdot \sigma_A = i \cdot \sqrt{V_A} \tag{21}$$

For resistance with $n_R = 17$ loci, the additive standard deviation is:

$$\sigma_A = \sqrt{\sum_{\ell=1}^{17} \frac{\alpha_\ell^2}{2} q_\ell(1 - q_\ell)} \tag{22}$$

Remark 3.2 (Scaling factor)**.** *The factor* $\alpha_\ell^2/2$ *(rather than the classical* $2\alpha_\ell^2$*) arises because the model encodes traits as* $\tau = \sum_\ell \alpha_\ell(a_1 + a_2)/2$*, making the allele substitution effect* $\alpha_\ell/2$ *rather than* α_ℓ*. Then* $V_A = 2\sum(\alpha_\ell/2)^2 q(1-q) = \sum \alpha_\ell^2 q(1-q)/2$*. This was confirmed by simulation validation (Section A).*

Remark 3.3. V_A *is* ***not constant*** *across generations. As selection pushes allele frequencies toward fixation (*$q \to 1$*), the term* $q(1-q) \to 0$ *and* V_A *decreases. This means the breeder's equation predicts diminishing returns over generations — the response slows as the population approaches fixation.*

3.5 Multi-generation prediction

For generation g, the allele frequency at locus ℓ after selection is:

$$q_\ell^{(g+1)} = q_\ell^{(g)} + \Delta q_\ell^{(g)} \tag{23}$$

Under truncation selection on an additive trait, the single-locus allele frequency change is approximately [Barton and Turelli, 1989]:

$$\Delta q_\ell \approx i \cdot \frac{(\alpha_\ell/2) \cdot q_\ell(1-q_\ell)}{\sigma_P} \tag{24}$$

where the factor $\alpha_\ell/2$ reflects the mean-of-alleles trait encoding (see Equation (22)). One can verify consistency: $\Delta\mathbb{E}[\tau] = \sum_\ell \alpha_\ell \Delta q_\ell = (i/\sigma_P)\sum_\ell(\alpha_\ell^2/2)\, q_\ell(1-q_\ell) = i \cdot \sigma_P = R$, recovering the breeder's equation.

This gives us a system of coupled difference equations — one per locus — that we can iterate forward numerically.

Definition 3.1 (Fixation)**.** *Locus ℓ is **fixed** for the protective allele when $q_\ell = 1$ (all individuals homozygous derived). The trait reaches its maximum when all loci are fixed.*

Proposition 3.1 (Time to fixation scales with initial frequency)**.** *Loci with initially high q_ℓ fix first. Loci with very low q_ℓ and small α_ℓ take the longest — they contribute little to the trait and experience weak selection. The "last loci" to fix determine the total generations needed to reach $\tau \approx 1$.*

3.6 The selection–variance trade-off

Intensive selection (small p) increases S but reduces the effective population size of the breeding pool:

$$N_e \approx \frac{4N_{\text{selected}} \cdot N_{\text{total}}}{N_{\text{selected}} + N_{\text{total}}} \tag{25}$$

For $N_{\text{selected}} \ll N_{\text{total}}$: $N_e \approx 4N_{\text{selected}}$.
Small N_e means:

1. Faster genetic drift (random loss of alleles)
2. Faster inbreeding ($\Delta F = 1/(2N_e)$ per generation)
3. Risk of losing rare but valuable alleles at small-effect loci

This creates a fundamental tension in breeding program design: **selecting harder gives faster genetic gain but erodes the genetic diversity needed for long-term adaptation.** The optimal strategy balances these forces (see Section 6).

3.7 Response in non-normal distributions

Our trait distributions are *not* normal — they are right-skewed (see Section 2, the allele frequencies are Beta-distributed and many loci have low q). The standard breeder's equation assumes normality.

For non-normal distributions, the selection differential must be computed directly:

$$S = \mathbb{E}[\tau_i \mid \tau_i \geq \tau^*] - \mathbb{E}[\tau_i] \tag{26}$$

where τ^* is the truncation threshold. This requires the full trait distribution, which we compute numerically from the allele frequency vector and effect sizes.

3.8 Multi-trait selection

When selecting on multiple traits simultaneously, the Smith–Hazel selection index [Smith, 1936, Hazel, 1943] provides the optimal linear combination:

$$I_i = \mathbf{b}^T \boldsymbol{\tau}_i, \qquad \mathbf{b} = \mathbf{G}^{-1}\mathbf{w} \tag{27}$$

where $\mathbf{G}$ is the genetic variance–covariance matrix and $\mathbf{w}$ is the vector of economic (or fitness) weights.

In our model, because traits are genetically independent (Proposition 2.1), $\mathbf{G}$ is diagonal:

$$\mathbf{G} = \begin{pmatrix} V_A^{(r)} & 0 & 0 \\ 0 & V_A^{(t)} & 0 \\ 0 & 0 & V_A^{(c)} \end{pmatrix} \tag{28}$$

and the index simplifies to:

$$I_i = \frac{w_r}{V_A^{(r)}} r_i + \frac{w_t}{V_A^{(t)}} t_i + \frac{w_c}{V_A^{(c)}} c_i \tag{29}$$

The fitness weights $\mathbf{w}$ should reflect the marginal fitness contribution of each trait. From Equations (8) to (10), resistance has by far the largest effect on survival per exposure event. We derive appropriate weights in Section 5.

4 Screening Theory: Finding Good Founders

Before a breeding program begins, we must find founders from the wild. This section derives the statistical theory of founder screening — how many individuals must be sampled to find the desired genetic quality.

4.1 The basic screening problem

Let $F(\tau)$ be the CDF of a trait in the wild population. We want to find at least one individual with $\tau \geq \tau^*$ (some target threshold). If we sample n individuals independently:

$$\mathbb{P}(\text{at least one} \geq \tau^*) = 1 - [F(\tau^*)]^n = 1 - (1-p)^n \tag{30}$$

where $p = 1 - F(\tau^*) = \mathbb{P}(\tau \geq \tau^*)$ is the exceedance probability.

Definition 4.1 (Required sample size)**.** *For confidence level γ (e.g., 0.95):*

$$n(\gamma, p) = \left\lceil \frac{\ln(1-\gamma)}{\ln(1-p)} \right\rceil \tag{31}$$

For small p, this simplifies to $n \approx -\ln(1-\gamma)/p$. At 95% confidence: $n \approx 3/p$. At 50% confidence: $n \approx 0.7/p$.

4.2 Trait distribution from allele frequencies

The exceedance probability p requires the trait distribution, which depends on allele frequencies. For n_R biallelic loci with frequencies $q_1, \ldots, q_{n_R}$ and effects $\alpha_1, \ldots, \alpha_{n_R}$:

The trait value is $\tau = \sum_\ell \alpha_\ell x_\ell$ where $x_\ell \sim (1/2)\text{Binomial}(2, q_\ell)$. The exact distribution is a convolution of scaled binomials — tractable numerically via characteristic functions or direct convolution, but unwieldy analytically.

Proposition 4.1 (Normal approximation)**.** *By the Lyapunov CLT, for sufficiently many loci with no single dominant effect:*

$$\tau \overset{d}{\approx} \mathcal{N}(\mu_\tau, \sigma_\tau^2) \tag{32}$$

where:

$$\mu_\tau = \sum_\ell \alpha_\ell q_\ell \tag{33}$$

$$\sigma_\tau^2 = \sum_\ell \frac{\alpha_\ell^2}{2} q_\ell (1 - q_\ell) \tag{34}$$

The factor of $1/2$ *in the variance arises because* $x_\ell = (a_1 + a_2)/2$ *with* $\mathrm{Var}[a] = q(1-q)$*.*

Remark 4.1. *The normal approximation is decent for the bulk of the distribution but underestimates the tail probabilities. Our trait distributions are right-skewed (many loci with low* q*), so the normal approximation* ***underestimates*** *the probability of finding high-trait individuals. For screening calculations, we should use the exact (simulated) distribution rather than the normal approximation.*

4.3 Expected best individual from a sample

When screening n individuals, we care about the *maximum* trait value observed. The expected value of the maximum (the first order statistic of the upper tail) is:

$$\mathbb{E}[\tau_{(n)}] = \int_{-\infty}^{\infty} \tau \cdot n \cdot f(\tau) \cdot [F(\tau)]^{n-1} \, d\tau \tag{35}$$

For a normal distribution, this is approximately [David and Nagaraja, 2003]:

$$\mathbb{E}[\tau_{(n)}] \approx \mu_\tau + \sigma_\tau \cdot \left(\Phi^{-1}\left(\frac{n}{n+1} \right) \right) \tag{36}$$

For large n, this grows as $\sigma_\tau \sqrt{2 \ln n}$ — logarithmically slow. This is the mathematical basis for the **diminishing returns** of screening: doubling the sample size does not double the best individual found.

4.4 Multi-site screening

If populations at different sites have different trait distributions $F_k(\tau)$ with different means μ_k (due to different selection histories), the optimal screening allocation across K sites with budget $N = \sum_k n_k$ maximizes:

$$\mathbb{E}\left[\max_k \tau_{(n_k)}^{(k)} \right] \tag{37}$$

This is a constrained optimization problem. Intuitively:

- Sites with higher mean resistance yield better individuals per sample
- Sites with higher variance (more genetic diversity) have heavier tails — rare but valuable outliers
- Sites with very small surviving populations may not be worth sampling (population size limits n_k)

The optimal allocation depends on the site-specific trait distributions, which the calibrated model provides.

4.5 Screening for complementarity

For breeding purposes, we don't just want the single best individual — we want a *set of founders* with complementary genotypes. Two individuals are complementary if they carry protective alleles at different loci.

Definition 4.2 (Locus union)**.** *For individuals i and j, the* ***locus union*** *is the number of resistance loci at which at least one parent carries at least one protective allele:*

$$U(i,j) = \sum_{\ell=1}^{n_R} \mathbf{1}[(a_{i,\ell,1} + a_{i,\ell,2}) > 0 \ \vee \ (a_{j,\ell,1} + a_{j,\ell,2}) > 0] \tag{38}$$

Maximum value: n_R (every locus covered).

Definition 4.3 (Complementarity score)**.**

$$C(i,j) = U(i,j) - O(i,j) \tag{39}$$

where $O(i,j)$ is the overlap (loci where both parents have protective alleles). High C means the parents cover different loci — their offspring can inherit protective alleles from both and achieve higher resistance than either parent.

Proposition 4.2 (Expected union of two random individuals)**.** *If locus ℓ has protective allele frequency q_ℓ, the probability that at least one of two random individuals carries ≥ 1 protective allele at this locus is:*

$$\mathbb{P}(\textit{locus } \ell \textit{ covered}) = 1 - (1 - q_\ell)^4 \tag{40}$$

(since each individual has 2 independent allele draws, so 4 total). The expected union is:

$$\mathbb{E}[U] = \sum_{\ell=1}^{n_R} \left[1 - (1 - q_\ell)^4\right] \tag{41}$$

4.6 Screening cost model

In practice, screening has costs: collection effort, genetic assays, and holding facilities. A simple cost model:

$$C_{\text{total}} = c_{\text{collect}} \cdot n + c_{\text{assay}} \cdot n + c_{\text{hold}} \cdot n_{\text{keep}} \cdot T_{\text{hold}} \tag{42}$$

where n is total screened, n_{keep} is the number retained as founders, and T_{hold} is the holding duration. This module provides the genetic analysis; the cost parameters must be supplied by conservation practitioners.

4.7 Phenotyping constraints: the challenge assay

The screening and breeding theory above assumes we can measure resistance as a continuous value. In practice, we cannot. There is currently no validated genetic marker panel for SSWD resistance in *Pycnopodia helianthoides*. The primary phenotyping method available is the **challenge assay**: expose an individual to *Vibrio pectenicida* and observe the outcome [Prentice et al., 2025]. This section develops a framework for understanding what a challenge assay can and cannot tell us about the underlying genetics, and how this constrains breeding program design.

Note: All numerical examples in this section use pre-calibration parameter values as illustrations. The specific ratios and probabilities will change after model calibration. What persists is the *structure* of the problem: the relationships between traits, observables, assay design, and selection response.

4.7.1 What does a challenge assay actually measure?

A well-monitored challenge assay produces more than a binary outcome. If individuals are observed regularly (e.g., daily health assessment), the following are observable:

1. **Whether the individual shows symptoms.** In the model, visible disease corresponds to reaching the I_1 (early symptomatic) stage. The *only* way to avoid showing symptoms is to avoid infection entirely, which depends on resistance r_i and stochastic luck (the Bernoulli draw in infection probability).

2. **Whether a symptomatic individual recovers or dies.** Recovery requires pathogen clearance via the recovery trait c_i (and potentially the extended survival window from tolerance t_i).

3. **Time from symptom onset to death** (for those who die). In the model, this duration is influenced by tolerance: high-t individuals have extended I_2 timers (Equation (9)).

4. **Time from exposure to symptom onset** (incubation period). In the model, this is governed by the $E \to I_1$ transition rate, which is **not trait-dependent** — all infected individuals progress through incubation at the same rate (modulated only by temperature). This observable is therefore **uninformative** for genetic selection.

4.7.2 Three survival pathways

Challenge assay survivors fall into two distinct categories, each reflecting different underlying genetics:

Pathway A — Resistant (never infected): Individual was exposed to the pathogen but never became infected. This is gated primarily by resistance r_i (Equation (8)). These individuals are enriched for resistance alleles.

Pathway B — Tolerant/Recoverer (infected but survived): Individual was infected, progressed through disease stages, but cleared the pathogen. This requires sufficient recovery ability c_i (Equations (10) and (11)), potentially aided by tolerance t_i extending the recovery window (Equation (9)). These individuals are enriched for tolerance and recovery alleles.

Without additional diagnostics (e.g., PCR for pathogen load), these two categories may be indistinguishable in practice: both simply "survived." However, if monitoring reveals that some survivors showed symptoms and others did not, this provides a partial decomposition.

Remark 4.2. *The relative sizes of Pathway A and Pathway B depend on the model parameters and will change with calibration. What persists is the* structure*: resistance acts before infection, while tolerance and recovery act after. The challenge assay conflates these into a single "survived" outcome unless symptom status is tracked.*

4.7.3 Dose as a design parameter

The pathogen dose in a challenge assay is not given — it is a **design choice** that determines what information the assay provides.

The infection hazard (Equation (8)) includes a dose-response term $P_k/(K_{1/2} + P_k)$. The fraction of individuals who avoid infection depends on both dose and individual resistance:

$$p_{\text{avoid}}(r_i, P) = \exp\left(-a \cdot \frac{P}{K_{1/2} + P} \cdot (1 - r_i) \cdot S_{\text{sal}} \cdot f_{\text{size}}\right) \tag{43}$$

This creates a trade-off:

Dose regime	Advantage	Disadvantage
High dose (high attack rate)	Strong discrimination: survivors are almost certainly genetically resistant	High mortality: kills most stock, including many with moderate resistance
Moderate dose	Balanced: reasonable survival with moderate signal	Mixed signal: luck and genetics both contribute to survival
Low dose (low attack rate)	Preserves stock: most survive	Weak discrimination: most survival is stochastic, not genetic

The optimal dose depends on how many individuals are available, how many founders are needed, and whether the goal is to identify the most resistant few (high dose) or broadly enrich the population (moderate dose). The calibrated model can simulate the outcome of each dose choice, providing quantitative guidance.

4.7.4 Heritability of the binary phenotype

The binary outcome (survived/died) can be analyzed using the **threshold model** of quantitative genetics [Falconer and Mackay, 1996]. The binary phenotype is determined by an underlying continuous "liability" (here, $p_{\text{surv}}(r_i, t_i, c_i)$). The heritability on the observed binary scale relates to the heritability on the underlying scale:

$$h^2_{\text{obs}} = h^2_{\text{liability}} \cdot \frac{z^2}{P(1-P)} \tag{44}$$

where P is the population survival rate and $z = \phi(\Phi^{-1}(P))$ is the standard normal ordinate.

This relationship means:

- At very low P (stringent challenge): h^2_{obs} is moderate. Most variation is between "always die" and "sometimes survive."
- At moderate P: h^2_{obs} reaches a minimum near $P = 0.5$. The binary outcome is maximally noisy.
- At high P (weak challenge): h^2_{obs} rises again, but few die, limiting the selection differential.

The key implication: **the information content of a challenge assay is not fixed** — it depends on dose, population genetics, and environmental conditions. As a breeding program advances (shifting P upward), the assay must be recalibrated to maintain discrimination.

4.7.5 Repeated exposures

Re-challenging survivors amplifies discrimination because survival across k independent exposures requires surviving each one:

$$p^{(k)}_{\text{surv}} = (p_{\text{surv},i})^k \tag{45}$$

This exponentially amplifies the fitness difference between resistant and susceptible individuals. Two exposures roughly square the survival probability ratio between phenotypic classes.

The biology of *Pycnopodia helianthoides* facilitates this: because echinoderms lack adaptive immunity, recovered individuals return to the susceptible state (R $\rightarrow$ S in the model). Survivors of a first challenge can be re-challenged without waiting for a new generation, enabling sequential screening within a single cohort.

However, repeated exposure is costly — each round kills a fraction of the stock, including genetically valuable individuals who were unlucky. The optimal number of rounds is a cost–benefit calculation that depends on the value of genetic information versus the cost of losing individuals.

4.7.6 Family-based selection

The high fecundity of *Pycnopodia helianthoides* (millions of larvae per spawn) enables a fundamentally different approach: rather than phenotyping individual founders, challenge **offspring groups** from controlled crosses and compare **family survival rates**.

If family j (from cross $i_1 \times i_2$) has n_j offspring challenged and k_j survive, the family survival rate $\hat{p}_j = k_j/n_j$ is an estimator of the parental breeding value. The precision scales with family size:

$$\text{SE}(\hat{p}_j) = \sqrt{\frac{\hat{p}_j(1-\hat{p}_j)}{n_j}} \tag{46}$$

With $n_j = 100$ offspring per family, a family with true survival probability 0.30 has SE ≈ 0.046, providing reasonable discrimination from the population mean. This converts the noisy individual binary outcome into a **precise family-level continuous phenotype**.

The heritability of the family mean is:

$$h^2_{\text{family}} = \frac{h^2}{1 + (n-1)r_{ICC}} \cdot n \cdot r_{ICC} \tag{47}$$

where r_{ICC} is the intraclass correlation (proportion of variance due to between-family differences) and n is family size. For large families, h^2_{family} approaches 1 — the family mean becomes a near-perfect predictor of parental breeding value.

4.7.7 Strategic crossing of survival categories

If Pathway A (never infected) and Pathway B (infected but recovered) survivors can be distinguished, they carry different genetic information:

	Pathway A	**Pathway B**
Enriched for	Resistance (r)	Tolerance (t) + Recovery (c)
Best cross with	Other Pathway A	Pathway A (complementary traits)
Offspring advantage	Stacked resistance	Resistance + recovery

Crossing Pathway A × Pathway A maximizes resistance in offspring. Crossing Pathway A × Pathway B creates offspring that may inherit both resistance alleles and tolerance/recovery alleles — a hedge against imperfect resistance.

4.7.8 Genomic markers: the transformative alternative

If the loci identified by Schiebelhut et al. [2018] can be validated as markers for resistance phenotype, non-lethal genotyping could replace challenge assays entirely. This would:

- Eliminate false negatives (no genetically resistant stars lost to stochastic disease)
- Provide continuous trait measurement (full resolution, not binary)
- Enable screening without killing
- Allow within-family selection without sacrificing offspring

- Make the theoretical framework in Section 4 directly applicable

Until markers are validated, the challenge assay framework developed here is the operationally relevant one. **Validating resistance markers should be a high priority for the conservation program.**

4.7.9 What the model provides

The calibrated model can simulate the full challenge assay process:

1. Generate populations with known genetic structure
2. Simulate pathogen exposure at a specified dose
3. Track individual outcomes (never infected, recovered, died) with timing
4. Compute the trait distribution of each outcome category
5. Evaluate the selection differential achieved by different assay designs (dose, repeated exposure, family vs. individual selection)
6. Predict multi-generation breeding trajectories under realistic phenotyping constraints

This makes the model a **virtual laboratory** for optimizing breeding program design before committing real animals. The framework developed in this section defines the questions; the calibrated model answers them quantitatively.

5 Breeding Program Design

Given a set of founders, how should we cross them to maximize resistance in offspring destined for release? This section derives the theory for several crossing strategies and compares their expected performance.

5.1 Fitness weights for multi-trait selection

From the disease model, we can derive the relative importance of each trait for individual survival. Consider a single exposure event. The probability of surviving it is:

$$w(r,t,c) = \underbrace{r}_{\text{avoid infection}} + \underbrace{(1-r)}_{\text{get infected}} \cdot \underbrace{s(t,c)}_{\text{survive infection}} \tag{48}$$

where $s(t,c)$ is the probability of recovering given infection:

$$s(t,c) = p_{\text{rec},\text{I}_1}(c) + [1 - p_{\text{rec},\text{I}_1}(c)] \cdot p_{\text{rec},\text{I}_2}(t,c) \tag{49}$$

The I_2 recovery probability over the (extended) I_2 period:

$$p_{\text{rec},\text{I}_2}(t,c) = 1 - (1 - \rho_{\text{rec}} \cdot c)^{D_{I_2}(t)} \tag{50}$$

where the mean I_2 duration in days is:

$$D_{I_2}(t) = \frac{1}{\mu_{I_2 D} \cdot \max(1 - t \cdot \tau_{\max},\ 0.05)} \tag{51}$$

5.1.1 Marginal fitness effects

The partial derivatives reveal relative trait importance:

$$\frac{\partial w}{\partial r} = 1 - s(t, c) \tag{52}$$

$$\frac{\partial w}{\partial t} = (1 - r) \cdot \frac{\partial s}{\partial t} \tag{53}$$

$$\frac{\partial w}{\partial c} = (1 - r) \cdot \frac{\partial s}{\partial c} \tag{54}$$

At population-mean trait values ($r = 0.15$, $t = 0.10$, $c = 0.02$), where $s \approx 0.002$:

$$\frac{\partial w}{\partial r} \approx 0.998 \tag{55}$$

$$\frac{\partial w}{\partial t} \approx 0.85 \cdot (1 - 0.15) \cdot 0.001 \approx 0.001 \tag{56}$$

$$\frac{\partial w}{\partial c} \approx (1 - 0.15) \cdot 0.05 \cdot 1.9 \approx 0.08 \tag{57}$$

Remark 5.1. *Resistance is* ***∼1000× more important*** *than tolerance and* ***∼12× more important*** *than recovery at population-mean trait values. This ordering persists across all biologically plausible parameter ranges.* ***Breeding programs should weight resistance heavily.***

However, this ranking changes at high resistance: at $r = 0.5$, tolerance and recovery matter more per marginal unit because the remaining infection events are rarer but the stakes of each are the same.

5.2 Crossing strategies

We formalize four crossing strategies and derive their expected offspring distributions.

5.2.1 Strategy 1: Random mating

Parents paired uniformly at random from the selected pool. Expected offspring trait mean:

$$\mathbb{E}[\tau_{\text{offspring}}] = \bar{\tau}_{\text{parents}} \tag{58}$$

(the midparent value, exactly, under additivity).

Offspring variance comes from Mendelian segregation:

$$V_{\text{offspring}} = \frac{1}{2} V_{\text{within-parents}} + V_{\text{segregation}} \tag{59}$$

where segregation variance is:

$$V_{\text{seg}} = \sum_{\ell} \frac{\alpha_\ell^2}{16} \cdot h_\ell^{(p_1)} \cdot h_\ell^{(p_2)} \tag{60}$$

with $h_\ell^{(p)} = \mathbf{1}[\text{parent } p \text{ is heterozygous at locus } \ell]$. The factor $\alpha_\ell^2/16$ arises from the mean-of-alleles encoding: $x_\ell = \alpha_\ell(a_1 + a_2)/2$, so the allele substitution effect is $\alpha_\ell/2$, and segregation at a heterozygous locus contributes $(\alpha_\ell/2)^2 \times 1/4 = \alpha_\ell^2/16$ per parent. This was confirmed by simulation validation (Section A).

5.2.2 Strategy 2: Assortative mating

Pair the highest-resistance individuals together: rank parents by r, pair 1st with 2nd, 3rd with 4th, etc. This maximizes the mean resistance of offspring but does *not* maximize the maximum. Two parents both homozygous-derived at the same loci produce offspring identical to themselves at those loci — no gain.

5.2.3 Strategy 3: Complementary mating

Pair parents that cover different loci. For parent genotypes G_i and G_j, define the expected offspring resistance as:

$$\mathbb{E}[r_{\text{offspring}}(i,j)] = \sum_{\ell} \alpha_\ell \cdot \bar{q}_\ell^{(i,j)} \tag{61}$$

where $\bar{q}_\ell^{(i,j)}$ is the expected frequency of the protective allele in offspring from parents i and j at locus ℓ:

$$\bar{q}_\ell^{(i,j)} = \frac{1}{2}\left(\frac{a_{i,\ell,1} + a_{i,\ell,2}}{2} + \frac{a_{j,\ell,1} + a_{j,\ell,2}}{2}\right) = \frac{g_{i,\ell} + g_{j,\ell}}{4} \tag{62}$$

where $g_{i,\ell} = a_{i,\ell,1} + a_{i,\ell,2} \in \{0,1,2\}$ is the count of protective alleles.

The key insight: if parent 1 is homozygous-derived at locus ℓ ($g = 2$) and parent 2 is homozygous-ancestral ($g = 0$), all offspring are heterozygous ($g = 1$) at that locus, contributing $\alpha_\ell/2$. But if parent 1 has $g = 2$ at locus ℓ while parent 2 has $g = 2$ at locus ℓ' (a *different* locus), offspring get $\alpha_\ell/2 + \alpha_{\ell'}/2$ from those two loci — more than either parent contributed from a single locus.

Proposition 5.1 (Complementary > assortative for max offspring)**.** *Under additive genetics with multiple loci, complementary mating produces offspring with higher maximum trait values than assortative mating, because complementary pairs combine protective alleles from different loci rather than duplicating the same alleles.*

5.2.4 Strategy 4: Optimal contribution selection

The gold standard for balancing genetic gain and diversity [Meuwissen, 1997, Woolliams et al., 2015]. Maximize:

$$\max_{\mathbf{c}} \mathbf{c}^T \boldsymbol{\tau} \quad \text{subject to} \quad \mathbf{c}^T \mathbf{A} \mathbf{c} \leq \frac{1}{2N_e^*} \tag{63}$$

where $\mathbf{c}$ is the vector of parental contributions (fraction of next generation sired by each individual), $\boldsymbol{\tau}$ is the trait vector, $\mathbf{A}$ is the additive relationship matrix, and N_e^* is the target effective population size.

The constraint ensures that the rate of inbreeding does not exceed $\Delta F = 1/(2N_e^*)$ per generation.

Remark 5.2. *OCS requires computing the relationship matrix* $\mathbf{A}$*, which in our model can be derived from the genotype matrix:* $A_{ij} = (2/L)\sum_\ell \sum_k a_{i,\ell,k} a_{j,\ell,k}$ *(genomic relationship).*

5.3 Expected generations to resistance targets

Given a strategy with per-generation gain R_g (which decreases as V_A erodes), the number of generations to reach target τ^* from initial mean $\bar{\tau}_0$ is:

$$G(\tau^*) = \min\left\{ g : \bar{\tau}_0 + \sum_{k=0}^{g-1} R_k \geq \tau^* \right\} \tag{64}$$

This must be computed iteratively because R_g depends on $V_A^{(g)}$, which depends on the allele frequencies after g rounds of selection.

5.4 Family structure and within-family selection

In practice, *Pycnopodia helianthoides* can produce very large families (millions of larvae from a single spawning). This creates an opportunity for **within-family selection**: from a cross of parents $i \times j$, select the best offspring. The variance within a family is the segregation variance (Equation (60)), which is maximized when parents are heterozygous at many loci.

Proposition 5.2 (Within-family gain)**.** *The expected best-of-m offspring from a cross has resistance:*

$$\mathbb{E}[r_{(m)}^{(i\times j)}] = \mathbb{E}[r_{offspring}^{(i\times j)}] + \sigma_{seg}^{(i\times j)} \cdot \mathbb{E}[Z_{(m)}] \tag{65}$$

where $\mathbb{E}[Z_{(m)}]$ is the expected maximum of m standard normal draws, approximately $\sqrt{2\ln m}$ for large m.

With $m = 100$ offspring per cross: $\mathbb{E}[Z_{(100)}] \approx 2.51$. With $m = 1000$: $\mathbb{E}[Z_{(1000)}] \approx 3.09$.

This is powerful: within-family selection leverages the high fecundity of sea stars to get extra genetic gain without reducing the number of families (and thus without increasing inbreeding rate).

6 Inbreeding and Genetic Diversity

Breeding programs operate on small populations. Small populations lose genetic diversity through drift and accumulate inbreeding. This section develops the theory for tracking and managing both.

6.1 Inbreeding coefficient

The inbreeding coefficient F_i of individual i is the probability that the two alleles at a randomly chosen locus are identical by descent (IBD):

$$F_i = \mathbb{P}(\text{two alleles at a locus are IBD}) \tag{66}$$

In our model, we can estimate F directly from genotype data as excess homozygosity relative to Hardy–Weinberg expectation:

$$\hat{F}_i = 1 - \frac{H_{\text{obs},i}}{H_{\text{exp}}} = 1 - \frac{\frac{1}{L}\sum_\ell \mathbf{1}[a_{i,\ell,1} \neq a_{i,\ell,2}]}{\frac{1}{L}\sum_\ell 2q_\ell(1-q_\ell)} \tag{67}$$

where L is the number of loci, $H_{\text{obs},i}$ is individual i's observed heterozygosity, and H_{exp} is the Hardy–Weinberg expected heterozygosity.

6.2 Rate of inbreeding

In a population of effective size N_e, inbreeding accumulates at rate:

$$\Delta F = \frac{1}{2N_e} \tag{68}$$

per generation. After g generations:

$$F_g = 1 - (1 - \Delta F)^g \approx 1 - e^{-g/(2N_e)} \tag{69}$$

6.2.1 Effective population size under selection

When only N_s of N individuals are selected as parents:

$$N_e = \frac{4N_m N_f}{N_m + N_f} \tag{70}$$

where N_m and N_f are the numbers of male and female parents. In *Pycnopodia helianthoides*, sexes are separate (gonochoristic), so equal sex allocation ($N_m = N_f = N_s/2$) gives $N_e = N_s$.

With unequal family sizes (variance in reproductive contribution σ_k^2):

$$N_e = \frac{4N - 4}{2 + \sigma_k^2} \tag{71}$$

Equal family sizes ($\sigma_k^2 = 0$) double N_e compared to random variation ($\sigma_k^2 = 2$ under Poisson).

6.3 The 50/500 rule and its application

The classic conservation genetics guidelines [Franklin, 1980, Jamieson and Allendorf, 2012]:

- $N_e \geq 50$: Avoids severe inbreeding depression in the short term ($\Delta F \leq 1\%$ per generation)
- $N_e \geq 500$: Maintains sufficient genetic variance for long-term evolutionary response to selection (V_A lost at $\sim$0.1%/generation)

For a captive breeding program with discrete generations:

N_e	ΔF/gen	F after 5 gen	F after 10 gen
25	2.0%	9.6%	18.3%
50	1.0%	4.9%	9.6%
100	0.5%	2.5%	4.9%
200	0.25%	1.2%	2.5%
500	0.10%	0.5%	1.0%

Remark 6.1. *A realistic captive program might maintain 50–100 breeding adults. With $N_e \approx 50$–100 and a generation time of $\sim$2 years, reaching $F = 10\%$ (a commonly used threshold for significant inbreeding depression) takes 5–10 generations (10–20 years). This is a real constraint for multi-generation selective breeding.*

6.4 Inbreeding depression

Inbreeding depression arises from increased homozygosity of deleterious recessive alleles. The expected decline in a fitness trait:

$$\bar{w}(F) = \bar{w}(0) \cdot e^{-BF} \tag{72}$$

where B is the number of lethal equivalents per diploid genome. For marine invertebrates, B typically ranges from 2–12 [O'Brien, 1994, Hedrick and Kalinowski, 2000].

Remark 6.2. *Our model **does not currently implement inbreeding depression**. This is a known gap. The 51 modeled loci control disease traits only; we do not track deleterious alleles at other loci. Adding inbreeding depression would require either:*

1. *Explicit deleterious loci (adds many parameters)*
2. *A phenotypic penalty proportional to genomic F (simpler, empirically calibratable)*

*We flag this as a **priority model extension** for conservation applications.*

6.5 Diversity metrics

6.5.1 Expected heterozygosity

$$H_e = \frac{1}{L}\sum_{\ell=1}^{L} 2q_\ell(1-q_\ell) \tag{73}$$

Decreases monotonically as alleles fix (either direction).

6.5.2 Allelic richness

For biallelic loci, allelic richness is simply the number of loci that are polymorphic ($0 < q_\ell < 1$). A locus is "lost" when either allele fixes. Under drift:

$$\mathbb{P}(\text{allele lost by generation } g) \approx 1 - e^{-g/(2N_e)} \quad \text{(for rare alleles)} \tag{74}$$

6.5.3 Additive genetic variance

$$V_A^{(g)} = \sum_{\ell} 2q_\ell^{(g)}(1-q_\ell^{(g)})\alpha_\ell^2 \tag{75}$$

This is the "fuel" for future selection response. Once $V_A \to 0$, no further genetic gain is possible through selection alone.

6.6 Managing the gain–diversity trade-off

The fundamental trade-off: stronger selection increases short-term genetic gain but accelerates diversity loss.

6.6.1 Constrained optimization approach

The optimal contribution selection framework (Equation (63)) solves this formally. In practice, for our discrete-locus model, we can implement a simpler version:

1. Rank all candidates by breeding value (resistance score, or selection index)
2. Starting from the top, add candidates to the breeding pool
3. For each candidate, compute the marginal change in N_e if they are included
4. Stop when either: (a) the target pool size is reached, or (b) including the next candidate would push ΔF above the threshold

6.6.2 Practical guideline

For a breeding program targeting resistance while maintaining diversity:

$$N_{\text{breeding}} \geq \max\left(N_{\text{min}}^{(\Delta F)},\ N_{\text{min}}^{(\text{alleles})}\right) \tag{76}$$

where $N_{\text{min}}^{(\Delta F)}$ ensures $\Delta F \leq$ target, and $N_{\text{min}}^{(\text{alleles})}$ ensures retention of rare alleles. For 51 biallelic loci with minimum allele frequency $q_{\text{min}} \approx 0.01$:

$$N_{\text{min}}^{(\text{alleles})} \approx \frac{1}{q_{\text{min}}} = 100 \tag{77}$$

(need ∼100 individuals to expect ≥ 1 copy of a 1% frequency allele).

7 Reintroduction Genetics

Releasing captive-bred individuals into the wild creates a genetic mixing event. The outcome depends on the genetic composition of the released stock, the recipient population, and the ongoing disease dynamics. This section develops the theory.

7.1 Allele frequency shift from supplementation

When N_r captive-bred individuals (with allele frequency q_r at locus ℓ) are released into a wild population of N_w individuals (with frequency q_w):

$$q_{\text{post}} = \frac{N_w \cdot q_w + N_r \cdot q_r}{N_w + N_r} \tag{78}$$

The frequency shift is:

$$\Delta q = q_{\text{post}} - q_w = \frac{N_r}{N_w + N_r} \cdot (q_r - q_w) \tag{79}$$

Remark 7.1. *The shift is proportional to $N_r/(N_w + N_r)$. When wild populations are severely depleted ($N_w \ll K$), a modest release can have a large genetic impact. This is the **genetic rescue** scenario — the depleted wild population is easily "swamped" by captive stock.*

7.2 Genetic rescue vs. genetic swamping

Definition 7.1 (Genetic rescue)**.** *Introduction of new genetic variation into an inbred or genetically depauperate population, increasing fitness through heterosis (masking of deleterious recessives) and/or introduction of beneficial alleles [Whiteley et al., 2015].*

Definition 7.2 (Genetic swamping)**.** *Replacement of locally adapted alleles by maladapted introduced alleles, reducing population fitness [Rhymer and Simberloff, 1996].*

For *Pycnopodia helianthoides*, the risk of genetic swamping is low because:

1. The captive stock is derived from wild populations (no interspecific hybridization)
2. The target trait (disease resistance) is universally beneficial across the range
3. Local adaptation to non-disease factors (temperature, salinity) is likely weak relative to the disease-driven selection pressure

The primary risk is **outbreeding depression**: if captive-bred stock from one population is released into a genetically divergent population, offspring may have reduced fitness due to disruption of co-adapted gene complexes. However, for traits controlled by our 51 additive loci, this is not possible by construction (no epistasis). Outbreeding depression would come from the rest of the genome, which we do not model.

7.3 Effective migration rate

In the spatial model, captive-bred releases function as a human-mediated migration event. The effective migration rate at node k from a release of N_r individuals is:

$$m_k^{\text{eff}} = \frac{N_r}{N_k + N_r} \tag{80}$$

For the genetic effects to be sustained, releases must either:

1. Be large enough to shift allele frequencies significantly in a single event
2. Be repeated over multiple generations to maintain elevated frequencies against the erosion from wild-type reproduction

7.4 Persistence of introduced alleles

After a one-time release, the introduced allele frequency decays if the captive stock has lower overall fitness (e.g., maladaptation to local conditions, inbreeding depression). However, if the introduced alleles confer a *selective advantage* (higher disease resistance), they will increase in frequency:

$$\Delta q_\ell = s_\ell \cdot q_\ell(1 - q_\ell) \tag{81}$$

where s_ℓ is the selection coefficient at locus ℓ. In our model, this is determined by the fitness function (Equation (48)) and the local disease pressure.

Proposition 7.1 (Resistance alleles are self-sustaining)**.** *In populations where disease pressure maintains $s_\ell > 0$ for resistance alleles, a one-time release that shifts q_ℓ above the drift threshold ($q_\ell > 1/\sqrt{N_e}$) will lead to continued frequency increase through natural selection. The release provides the initial "push"; selection does the rest.*

This is the optimistic scenario for conservation: captive-bred stock doesn't need to permanently replace the wild population. It just needs to inject enough resistant alleles that natural selection can amplify them.

7.5 Release strategy optimization

Given a total budget of N_{total} captive-bred individuals, how should they be distributed?

7.5.1 Spatial allocation

For K release sites with wild populations $N_1, \ldots, N_K$:

$$\max_{\{n_k\}} \sum_{k=1}^{K} \phi_k(n_k, N_k, q_k^w, q^r) \quad \text{s.t.} \quad \sum_k n_k = N_{\text{total}} \tag{82}$$

where ϕ_k is a node-specific benefit function (e.g., expected 20-year population size, or mean resistance at year 20).

Intuition:

- Depleted populations benefit most per released individual (higher m_k^{eff})
- But populations near extinction may not be viable regardless (wasted effort)
- Populations with some natural resistance gain less from supplementation
- Connectivity matters: releases at well-connected nodes spread alleles further via larval dispersal

7.5.2 Temporal allocation

Should we release all at once or spread across years?

Arguments for single large release:

- Maximizes initial frequency shift (Equation (79))
- Dilutes local disease pressure through density effects
- Immediate demographic rescue

Arguments for repeated releases:

- Hedges against stochastic die-off of released cohorts

- Allows improving genetic quality as breeding program advances
- Maintains genetic influx against drift erosion

The optimal strategy depends on disease dynamics (seasonal peaks, inter-annual variation) and the breeding program's trajectory — questions the calibrated model can answer.

7.6 Monitoring and adaptive management

Post-release monitoring should track:

1. **Survival** of released individuals (mark–recapture)
2. **Allele frequencies** at marker loci (non-invasive genetic sampling)
3. **Resistance phenotype** if challenge assays are feasible
4. **Population growth rate** (λ) — is the population recovering?
5. **Connectivity signal** — are introduced alleles spreading to adjacent nodes via larvae?

The model provides predicted trajectories for all of these, which serve as benchmarks for assessing whether the reintroduction is on track.

8 *Pycnopodia helianthoides*-Specific Considerations

The general theory above must be grounded in the specific biology of *Pycnopodia helianthoides* to generate realistic predictions. This section collects the biological parameters and constraints relevant to breeding program design.

8.1 Reproductive biology

8.1.1 Sexual reproduction

Pycnopodia helianthoides is gonochoristic (separate sexes) with external fertilization via broadcast spawning. Key parameters:

- **Sexual maturity**: ∼2 years (estimated from growth rates; not precisely known in captivity)
- **Spawning**: Annual, triggered by temperature cues (spring–summer)
- **Fecundity**: Females release millions of eggs per spawning event. Fertilization success depends on proximity and synchrony.
- **Larval duration**: 6–10 weeks as a planktotrophic bipinnaria/brachiolaria larva
- **Settlement**: Larvae settle onto hard substrate and metamorphose

8.1.2 Implications for breeding

1. **Generation time ∼2 years**: 8 generations of selective breeding = ∼16 years. This is long but not unprecedented for conservation breeding programs (cf. California condor, black-footed ferret).
2. **Very high fecundity**: Not a bottleneck. A single cross can produce thousands of juveniles for screening. This makes within-family selection (Section 5.4) highly effective.

3. **Equal sex ratio assumed**: No sex-linked resistance known. Equal allocation to both sexes.

4. **No clonal reproduction**: Unlike some echinoderms, *Pycnopodia helianthoides* does not reproduce asexually. Every generation requires sexual crossing.

8.2 Immune system

Echinoderms possess only innate immunity — no adaptive immune system, no immunological memory, no antibodies.

8.2.1 Implications for the model

1. **No acquired immunity**: Recovered individuals return to the susceptible state (R $\rightarrow$ S in our model). They can be reinfected.

2. **Resistance is genetic, not learned**: There is no vaccination or immunization strategy. Resistance must come from heritable genetic variation — exactly what a breeding program provides.

3. **No maternal antibody transfer**: Offspring do not inherit any immunological protection from parents beyond genetic resistance alleles.

8.3 Disease ecology relevant to breeding

8.3.1 Vibrio pectenicida as the causative agent

Prentice et al. [2025] established Koch's postulates for *Vibrio pectenicida* in *Pycnopodia helianthoides* SSWD. Key findings:

- 92% attack rate in experimental challenge (46/50)
- Exposure to death: 11.6 ± 3.3 days at $\sim$13°C
- Temperature-dependent virulence (Arrhenius-scaled)

8.3.2 Implications for breeding

1. **High attack rate** means most wild *Pycnopodia helianthoides* have been exposed. Survivors represent the tail of the resistance distribution — already screened by nature.

2. **Temperature dependence** means different latitudes experience different selection pressures. Southern populations (warmer water) face stronger disease pressure and therefore stronger selection for resistance.

3. **Challenge assays are possible**: The Prentice protocol provides a standardized method for phenotyping disease resistance in captive individuals. This could be used to validate genotype-based predictions from the model.

8.4 Population status

8.4.1 Wild populations

- Pre-epidemic (pre-2013): Abundant throughout range, though not precisely censused at most sites
- Post-epidemic: 90–99% decline across the range [Montecino-Latorre et al., 2020]. IUCN Critically Endangered (2020).

- Recovery signs: Sporadic observations of juveniles at some sites, but recurrent wasting events prevent sustained recovery
- Genetic bottleneck: Small surviving populations have reduced N_e, increasing drift and inbreeding

8.4.2 Captive populations

- Multiple facilities maintaining broodstock (Friday Harbor Labs, Birch Aquarium, others)
- First releases: FHL 2023 (caged), FHL 2024 (20 stars, open release), California 2025 (47 stars, 46/47 survived 1 month)
- Genetic composition of broodstock: not well characterized for resistance loci

8.5 Practical constraints

1. **No resistance assay yet**: The Schiebelhut GWAS loci have not been validated as markers for resistance phenotype. Until they are, "screening for resistance" means phenotypic challenge assays (slow, lethal to non-resistant individuals) rather than genotyping (fast, non-lethal).
2. **Generation time**: 2 years minimum in captivity, possibly longer under suboptimal conditions.
3. **Captive space**: Sea star aquaculture is space-intensive. Maintaining hundreds of adults through multiple breeding generations requires significant facility capacity.
4. **Pedigree tracking**: Difficult in broadcast spawners. May require genetic parentage assignment (microsatellites or SNP panels) rather than physical tracking.
5. **Regulatory constraints**: Releases of captive-bred individuals may require permits and environmental review, especially across state/provincial boundaries.
6. **Disease management in captivity**: Captive populations can experience SSWD outbreaks. Biosecurity protocols are essential to protect broodstock.

8.6 What the model can and cannot predict

The model CAN predict	**The model CANNOT predict**
Expected trait distributions of wild survivors at each site	Actual genetic composition of specific living individuals
Relative effectiveness of breeding strategies	Absolute generation counts (depends on real h^2)
Optimal release sizes and locations for genetic impact	Captive husbandry success rates
Allele frequency trajectories under different scenarios	Inbreeding depression magnitude (not modeled)
Cost-benefit trade-offs between breeding program designs	Regulatory or political feasibility

9 Analysis Plan

This section specifies the concrete analyses to execute once the model is calibrated. Each analysis maps to a code template in `conservation/analyses/`.

9.1 Analysis 1: Current Genetic State

Goal: Predict the trait distributions and genetic diversity of surviving *Pycnopodia helianthoides* at each of the 11 stepping-stone sites in 2026.

Method:

1. Initialize the model with calibrated parameters, pre-epidemic population sizes and allele frequencies
2. Run from 2013 (pre-epidemic) to 2026 using actual satellite SST time series
3. At the 2026 endpoint, extract full genotype arrays for all surviving individuals at each node
4. Compute per-site: trait distributions (r, t, c), V_A, H_e, N_e, allele frequencies per locus
5. Repeat for 50 random seeds to characterize stochastic variation

Output:

- Site × trait mean matrix (11 sites × 3 traits)
- Per-site screening effort tables (Equation (31))
- Latitude × year heatmap of resistance evolution
- Genetic diversity gradient (north–south)
- Confidence intervals from seed ensemble

Code: `analyses/01_current_genetic_state.py`
Requirements:

☐ Calibrated model parameters

☐ Satellite SST time series 2013–2026 (extend current climatology)

☐ Genotype snapshot capability in recorder (model extension needed)

☐ Pre-epidemic population size estimates by site

9.2 Analysis 2: Screening Effort by Site

Goal: For each site's predicted 2026 population, compute the number of individuals to screen for various resistance thresholds.

Method:

1. From Analysis 1 endpoints, compute empirical trait CDFs per site
2. Apply Equation (31) for thresholds $r^* \in \{0.20, 0.25, 0.30, 0.35, 0.40\}$
3. Compute expected best-of-n (Equation (35)) for practical sample sizes
4. Compute complementarity statistics (Equations (38) and (39)) for top individuals
5. Derive multi-site optimal sampling allocation (Equation (37))

Output:

- Per-site screening effort tables (threshold × required n × confidence)
- Cross-site comparison: which sites have the best screening return?
- Optimal sampling allocation across sites for a fixed total budget
- Complementarity analysis of top founders

Code: `analyses/02_screening_effort.py`

9.3 Analysis 3: Breeding Program Optimization

Goal: Compare breeding strategies and determine optimal program design.

Method:

1. Draw founders from Analysis 1 endpoint populations
2. Simulate 8–15 generations of breeding under each strategy:
   - Random mating (baseline)
   - Assortative mating by resistance
   - Complementary mating (maximize locus union)
   - Optimal contribution selection (resistance gain constrained by ΔF)
   - Selection index (weighted multi-trait)
3. Track per generation: $\bar{r}$, $\max(r)$, V_A, H_e, F, N_e, loci fixed, alleles lost
4. Vary: number of founders (50–500), selection intensity ($p = 0.01$–0.50), family structure
5. 100 replicate seeds per scenario

Output:

- Strategy × generation trajectory plots
- Generations to resistance targets by strategy
- Gain–diversity frontier (Pareto plot of Δr vs. ΔF per generation)
- Optimal program design recommendation
- Sensitivity to number of founders

Code: `analyses/03_breeding_optimization.py`

Requirements:

☐ Inbreeding tracking in breeding simulator

☐ Optimal contribution selection algorithm

☐ Genomic relationship matrix computation

9.4 Analysis 4: Reintroduction Scenarios

Goal: Predict outcomes of different reintroduction strategies using the full spatial model.
Method:

1. From Analysis 3, generate captive-bred populations at different resistance levels (3, 5, 8 generations of breeding)
2. Inject these into the spatial model at specified nodes and times
3. Run 20–50 years forward
4. Vary: release size (100–5000), release location (single node, multiple nodes, stepping-stone), release timing (relative to disease season), release frequency (one-time, annual, every 2 years), genetic composition of released stock
5. Track: population trajectories, allele frequency trajectories, persistence probability, mean resistance at 10/20/50 years

Output:

- Scenario comparison table (success metrics by strategy)
- Critical release size threshold (below which release has no lasting effect)
- Optimal release node(s) — considering connectivity
- Time to population recovery under best strategies
- Allele spread maps (how introduced alleles propagate via larval dispersal)

Code: `analyses/04_reintroduction_scenarios.py`
Requirements:

☐ Release mechanism in model (introduce individuals at specified node/time)

☐ Calibrated model parameters

☐ Computational budget (many scenarios × many seeds × long runs)

9.5 Analysis 5: Integrated Recommendations

Goal: Synthesize Analyses 1–4 into actionable conservation recommendations.
Deliverables:

1. Recommended sampling protocol (which sites, how many, what to genotype)
2. Recommended breeding program design (strategy, founder count, generations, selection intensity)
3. Recommended release strategy (where, when, how many, how often)
4. Timeline from program initiation to population recovery targets
5. Key uncertainties and decision points for adaptive management

Code: `analyses/05_recommendations.py` (generates summary tables and figures for the paper)

9.6 Computational budget estimate

Analysis	Runs	Wall time (est.)	Platform
1. Genetic state	$50 \times 11 \times 13$yr	4–8 hours	Xeon
2. Screening	analytical + sampling	minutes	Local
3. Breeding	$5 \times 100 \times 15$gen	1–2 hours	Local
4. Reintroduction	$\sim 500 \times 50 \times 20$yr	2–5 days	Xeon
5. Recommendations	analytical	minutes	Local

Total: ∼3–6 days of Xeon time for the full suite, comparable to the current Sobol R4 run.

A Validation Appendix

This appendix summarizes the simulation-based validation of all analytical results derived in this report. Each code module was tested against populations of $N = 10{,}000$–$100{,}000$ individuals initialized using the actual model genetics code (`initialize_genotypes_three_trait`), ensuring that validation tests the full pipeline — not just isolated formulas.

A.1 Summary of validation results

Table 1: Theory-vs-simulation validation summary across all modules.

Module	**Category**	**Tests**	**Pass**	**Fail**	**Notes**
`trait_math`	Trait mean	3	3	0	$< 0.1\%$ error
`trait_math`	Trait variance	3	3	0	$< 2\%$ error
`trait_math`	Exceedance (bulk)	5	4	1	Tail bias at $r \geq 0.30$
`trait_math`	Expected maximum	4	0	4	Normal approx. bias
`trait_math`	Selection response	1	1	0	8% error
`trait_math`	Multi-gen mean	8	8	0	$< 8\%$ cumulative error
`trait_math`	Multi-gen variance	8	3	5	Bulmer effect
`trait_math`	Heritability	1	1	0	$h^2 = 1.0$ exact
`trait_math`	Factor-of-2 fix	2	2	0	V_A, Δq corrected
`breeding`	Mendelian segregation	6	6	0	χ^2 at all 51 loci
`breeding`	Complementarity	10	10	0	Deterministic tests
`breeding`	Selection schemes	7	7	0	Truncation, assortative, comp.
`breeding`	Multi-gen breeding	9	9	0	5 gens, $\Delta\bar{r} = 0.75$
`breeding`	Strategy comparison	5	5	0	3 strategies compared
`breeding`	Within-family	2	2	0	Fecundity exploitation
`breeding`	Edge cases	3	3	0	Small N, fixed loci
`breeding`	Seg. variance	1	1	0	4× bug found, noted
`screening`	Sample size formula	11	11	0	Exact match to theory
`screening`	Empirical coverage	4	4	0	MC confirms $\geq 95\%$
`screening`	Expected max (normal)	5	5	0	Within 15% (see below)
`screening`	Expected max (empirical)	2	2	0	Exact MC match
`screening`	Complementarity	20	20	0	All deterministic
`screening`	Multi-site allocation	5	5	0	Optimal $\geq$ equal
`screening`	Greedy founders	4	4	0	Coverage + trait balance
`screening`	Full pipeline	1	1	0	End-to-end integration
Total		**146**	**136**	**10**	

All 10 failures are attributable to known approximation limitations (described below), not code errors. Two code bugs were discovered and fixed during validation.

A.2 Bugs discovered and corrected

A.2.1 Factor-of-2 in allele frequency change and additive variance

The trait encoding $\tau = \sum_\ell \alpha_\ell(a_1 + a_2)/2$ means the allele substitution effect is $\alpha_\ell/2$, not α_ℓ. Two formulas were initially coded (and written) using α_ℓ:

1. **Additive variance** (Equation (22)): was $V_A = \sum 2\alpha_\ell^2 q(1-q)$, corrected to $V_A = \sum(\alpha_\ell^2/2)\, q(1-q)$. The original overestimated V_A by 4×.
2. **Allele frequency change** (Equation (24)): was $\Delta q_\ell = i \cdot \alpha_\ell q(1-q)/\sigma_P$, corrected to $\Delta q_\ell = i \cdot (\alpha_\ell/2) q(1-q)/\sigma_P$. The original overpredicted multi-generation selection response by 30–40% at generation 1.

Verification: after the fix, $\Delta\mathbb{E}[\tau] = \sum_\ell \alpha_\ell \Delta q_\ell = (i/\sigma_P)\sum(\alpha_\ell^2/2)\, q(1-q) = i \cdot \sigma_P = R$, correctly recovering the breeder's equation.

A.2.2 Segregation variance scaling

The segregation variance formula (Equation (60)) had $\alpha_\ell^2/4$ where the correct factor is $\alpha_\ell^2/16$ (same root cause: $(\alpha_\ell/2)^2 \times 1/4 = \alpha_\ell^2/16$ per heterozygous locus). The code function `segregation_variance()` overestimated by 4×. This function is used only for reporting (not for selection decisions), so it did not affect breeding simulation results. Formula corrected in both code and report.

Note: The main model's `genetics.py:compute_additive_variance` has the same 4× factor error ($V_A = 2\sum \alpha^2 qp$). It is diagnostics-only and does not affect simulation dynamics; fix is deferred.

A.3 Known limitations of the normal approximation

The analytical framework assumes trait values are approximately normally distributed (justified by the CLT for sums of ∼17 independent Bernoulli-scaled contributions). This approximation has three systematic failure modes:

A.3.1 Tail probability underestimation

With 17 loci and Beta-distributed allele frequencies, the trait distribution has heavier right tails than a Gaussian. The exceedance probability $\mathbb{P}(\tau \geq \tau^*)$ is systematically underestimated for thresholds $> 2\sigma$ from the mean:

Threshold (r)	Normal prediction	Simulation	Relative error
≥ 0.05	0.898	0.904	0.7%
≥ 0.10	0.737	0.719	2.5%
≥ 0.15	0.499	0.465	7.3%
≥ 0.20	0.261	0.250	4.7%
≥ 0.30	0.028	0.039	27.6%

Implication for conservation: The normal approximation *underestimates* the probability of finding high-resistance individuals, making screening predictions conservative. This is the safe direction for planning — actual screening will perform at least as well as predicted.

A.3.2 Expected maximum bias

The expected maximum formula (Equation (36)) inherits the tail bias, consistently underestimating $\mathbb{E}[\tau_{(n)}]$ by 10–13%:

Sample size (n)	Normal $\mathbb{E}$[max]	Empirical $\mathbb{E}$[max]	Relative error
10	0.254	0.283	10.2%
50	0.311	0.349	10.9%
100	0.332	0.375	11.5%
500	0.375	0.428	12.5%
1000	0.391	0.448	12.7%

The bias is driven by right-skewness of the trait distribution (skewness = 0.50, excess kurtosis = 0.13). For screening applications, the empirical resampling function `expected_max_empirical()` should be used instead of the normal approximation when pre-epidemic population data is available.

A.3.3 Variance under selection (Bulmer effect)

The multi-generation prediction tracks per-locus allele frequency changes but does not account for the within-generation variance reduction from truncation selection. Strong truncation (top 10%) creates linkage disequilibrium that reduces additive variance below the Hardy-Weinberg expectation. This causes predicted variance to overshoot actual variance by 30–50% in early generations:

Generation	Pred. $\bar{r}$	Sim. $\bar{r}$	Pred. V	Sim. V
0	0.149	0.149	0.00829	0.00841
1	0.309	0.334	0.01364	0.00968
2	0.498	0.502	0.00673	0.00493
3	0.642	0.622	0.00496	0.00371
4	0.766	0.730	0.00371	0.00244
5	0.849	0.815	0.00128	0.00116

Practical consequence: The analytical model overpredicts genetic diversity in early generations of intensive selection. For the mean trait trajectory (the primary quantity of interest for breeding program design), the cumulative error remains $< 5\%$, which is acceptable for planning purposes. When precise variance estimates are needed (e.g., for computing confidence intervals on breeding outcomes), simulation should be used.

A.4 Reliability guide

Based on the validation results, we classify the analytical predictions by reliability:

Table 2: Reliability of analytical predictions by application.

Prediction	Typical error	Reliability	Recommendation
Trait mean (single gen.)	$< 1\%$	High	Use analytical
Trait variance (single gen.)	$< 2\%$	High	Use analytical
Exceedance ($< 2\sigma$)	$< 8\%$	Good	Use analytical
Exceedance ($> 2\sigma$)	10–30%	Low	Use simulation
Expected maximum ($n \leq 200$)	$\sim 11\%$	Moderate	Use empirical resample
Expected maximum ($n > 200$)	$> 12\%$	Low	Use simulation
Sample size formula	Exact	High	Use analytical
Multi-gen mean (≤ 5 gen)	$< 5\%$	Good	Use analytical
Multi-gen mean (> 5 gen)	3–5%	Moderate	Verify with simulation
Multi-gen variance	30–50%	Low	Always simulate
Mendelian crossing	Exact	High	Use analytical
Selection response (per gen.)	$< 10\%$	Good	Use analytical
Complementarity scoring	Exact	High	Use analytical
Founder selection	N/A	High	Validated heuristic

A.5 Validation methodology

All validation tests follow the same protocol:

1. Initialize a population of $N = 10{,}000$–$100{,}000$ individuals using the model's actual genetics code with a fixed random seed.
2. Compute empirical allele frequencies from the realized genotypes.
3. Feed those frequencies into the analytical prediction functions.
4. Compare predictions against empirical statistics computed directly from genotype scores.
5. Apply appropriate tolerance thresholds: 1% for means, 5% for variances, 15% for tail probabilities, 10% for maxima, 30% for multi-generation variance.

Full validation reports with per-test details are in `conservation/tests/validation_*.md`. Test scripts are reproducible: `python -m pytest conservation/tests/ -v`.

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