Adaption Under Captive Breeding, and How to Avoid It

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Motivation for this talk

- Recently, and largely in conjunction with the request for federal listing of the eastern migratory population of the monarch as a threatened species, a group of monarch biologists have made a number of claims about the genetic risks of releasing reared monarchs
- While these claims are, at best, without merit, often contradictory, and appear to be policy-, not scientifically-, driven, they do raise some important issues for butterfly breeders
  - Specifically, when is it safe to release?

Outline

- Genetic divergence
  - Population structure
  - Adaptive vs. neutral divergence
- Genes in populations
  - Misconceptions
  - Selection vs. mutation, migration
  - Inbreeding
- Conditions for adaption/inbreeding in captivity
  - Conditions for its occurrence
  - Best practices

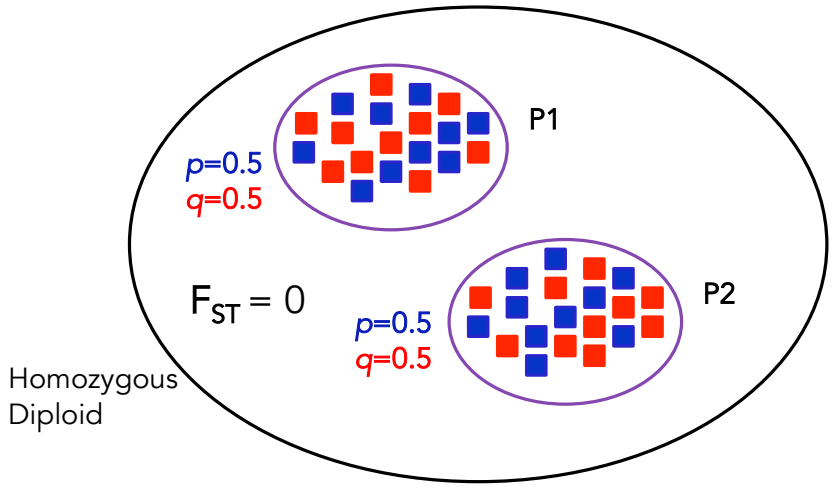
Issue one: Protecting the local ecology

- One should not release exotic species (those not native to an area) that are capable of breeding without a great deal of study and caution.
  - Little risk with tropical (non-overwintering) species in snow-belt areas
  - However, ecological interactions are unpredictable, and hence any such releases should be avoided, except under extremely carefully monitored / regulated conditions

Issue two: Protecting genetically distinct subpopulations

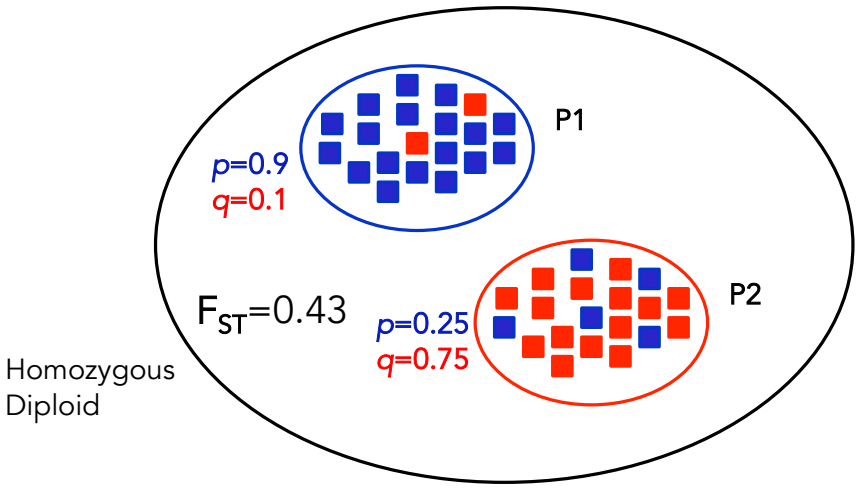
- When a species is composed of a number of genetically distinct isolated populations, releases should only involve those that match the genetic subpopulation in the area
  - How can we detect such population structure?
  - Standard measure is Sewall Wrights F_{ST} statistic, which measures the fraction of variation due to between-species differences
  - Can this estimate using molecular markers.

Graphical example of F_{ST}



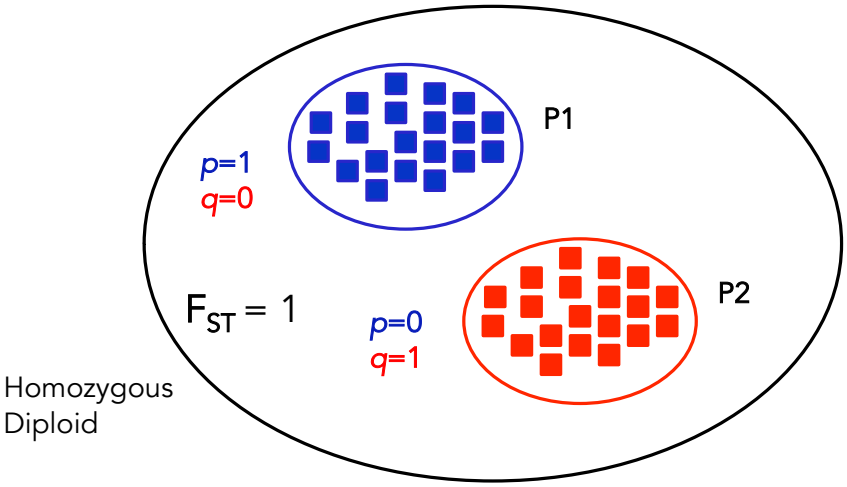
No population differentiation

Graphical example of F_{ST}



Strong population differentiation

Graphical example of F_{ST}



Complete population differentiation

- F_{ST} is a reasonable, but not ideal, measure of genetic differentiation
- Populations can differ entirely from genetic drift, the random sampling of alleles with no impact on fitness (this is what F_{ST} tries to measure)
  - Non-adaptive divergence
- They can, more importantly, also differ because of genetic adaption to specific conditions, which may involve far fewer genes
  - Localized adaptation
- Major phenotypic divergences can be either neutral or adaptive

Interplay of evolutionary forces

- Mutation, selection, and genetic drift can all conspire to cause genetic divergence between isolated populations
- However, a sufficient amount of migration can overpower these forces, making species divided into distinct demes still a single genetic unit
- Wright's result $F_{ST} = 1/(1+4Nm)$, where m = migration rate. Hence, ~ 10 migrants per generation between subpopulations is enough to prevent divergence under drift.

Key is strong migration

- A widespread population that displays strong migration, especially back to a small set of overwintering populations, is unlikely to have strong population structure, as any localized adaption gets randomized each year in the overwintering mating pool
- This is especially true for weedy species that have a fairly wide host range (i.e., feed on more than a handful of plants) and hence are adaptive to living under very broad conditions

Candidates for safe release

- Very broad geographic range with little to no population structure
- Migrate into an area from some distant overwintering source pool
- Are weedy in the sense of having either a wide range of hosts or feed on host plants that are themselves weedy

Safe candidates for release essentially anywhere

- Monarch
- Vanessa (cardui, annabella, virginensis, atalanta) – Painted ladies
- Argaulis vanillae -- Gulf fritillary
- Phoebis sennae – cloudless sulphur
- A number of others

Monarch population structure

- In the 1980's Chip Taylor (Kansas) urged that eastern monarchs should not be shipped west, and vise-versa. His concern was that these were genetically-isolated populations, and such transfer would imperil a population
- However, a number of recent studies (from STRs to genomic data) show no genetic distinction between eastern and western populations.
  - One reason, migrants (esp. in Arizona) can go to either California or Mexican overwintering sites, with this gene flow randomizing any potential local adaption (remember the ~ 10 rule)

Genetic risks

- Taylor was the first to suggest a genetic risk, and more recently Taylor and his colleagues have suggested a risk from reared monarchs.
- What do they claim and is it supported?
- As way of background, some introductory population genetics is needed.

Population genetics

- A genetic locus potentially carries a number of different alleles, and population genetics studies how evolutionary forces (drift, selection, mutation, migration) change these frequencies
- A number of serious misconceptions exist about how these forces interact

Misconception 1: The "contagion" model of deleterious alleles

- The idea that a deleterious mutation introduced (by mutation or migration) will spread.
- This was Taylor's concern, a less-than-fit allele for the eastern population introduced from the west would nonetheless spread
- Quite the opposite. Deleterious alleles are quickly removed from the population

Selection vs. drift or migration

- Suppose allele A reduces fitness by some amount s (aa fitness = 1, Aa fitness = 1-s)
- Such an allele is quickly lost from the population
- If it is constantly introduced (either by migration at rate m or mutation at rate u), its equilibrium frequency is ~ s/m or ~ s/u
- Hence, a strongly-deleterious allele (s ~ 0.05), requires a high migration rate (> 5% of the population are new migrants) for it to reach any frequency. Once migration is stopped, it rapidly declines to zero

Misconception 2: Bad Phenotypes mean bad genotypes

- P = G + E, Phenotype = Genotype plus Environmental value. If P is small because of a bad E, does not matter for its offspring, as (a part of) G is what is passed onto offspring.
- Further, if a phenotype has low fitness, by definition it leaves very few (if any) offspring

- "Lower fitness of captive bred animals can result purely from environmental causes, even in the absence of any genetic effects like inbreeding or selection. If animals being released are small …, this would not be helping monarch conservation efforts"
  - Dr. Sonia Aaltizer
  - http://akdavis6.wix.com/monarchscience#!What-everyone-needs-to-know-about-rearing-monarchs-from-a-science-standpoint/cy97/55e3736b0cf28ffc7eec64ac
- Incorrect. Fallacy that a small phenotype due to a negative environmental effect means a transmissible small genetic effect.

Misconception 3: The spread of inbreeding

- Inbreeding, the mating of close relatives is generally to be avoided in outcrossing species due to inbreeding depression.
- Inbreeding level F = Prob(both alleles are idb at a random locus)
  - Typically want F < 5-10%
- In a population of (effective) size N, inbreeding accrues at a rate of 1/(2N) per generation, or t/(2N) for t generations

Inbreeding (cont)

- Inbred individuals tend to have lower fitness.
- F ~ t/(2N), for N = 100, solving 0.05 = t/(2N) or t = (2N)*0.05 = 0.1*N
  - Hence, if your population size is 100, about 10 generations with give you F = 0.05
  - In a closed population, inbreeding continues to increase
  - However, if even a small fraction of wild material introduced each generation, inbreeding does not accumulate to any significant levels
  - m > 1/(2N)

The "spread" of inbreeding

- The coefficient of coancestry (θ) between two individuals measures the expected level of inbreeding in their offspring.
- If we cross two inbred individuals, the offspring is only inbred if their $\theta > 0$
- Hence, if inbred individuals are released into the wild, they most likely mate with a wild individual, in which case $\theta \sim 0$, and inbreeding goes away, rather than spreading
  - If $\theta \sim 0$, inbreeding in offspring ~ 0

Concerns about Mass-rearing and Selling of Monarchs By Sonia Altizer, Lincoln Brower, Elizabeth Howard, and Karen Oberhauser

2. Genetic diversity The loss of genetic diversity among wild monarchs is also a concern. We don't know how many parents are contributing to the genetic stock of any given purchase, and it seems likely that breeders will share stock to augment their breeding colonies. Thus it is very possible that many of the released monarchs could be related. The release of large numbers of individuals with low genetic diversity could contribute to further declines due to inbreeding depression.

http://www.learner.org/jnorth/tm/monarch/conservation_action_release.html

Reflects a deep ignorance of population-genetics

Misconception 4: Deleterious alleles will spread if selection is stopped

- Deleterious alleles do exist in nature, as mutation constantly generates them.
- However, their frequencies are very low (on the order of u/s ~ 1/1000 or smaller) as u ~ 10^{-6}.
  - More deleterious, the lower their frequency.
  - Hence, a small random sample from a population likely does not contain many deleterious mutations (~ one)
  - If selection is completely relaxed (very unlikely, even under careful rearing), alleles now under drift
  - The probability they significantly increase in frequency is proportional to their starting frequency, which is expected to be very low.
  - Hence, very unlikely to spread

Misconception 4: Deleterious alleles will spread if selection is stopped

- Rearing essentially bypasses natural selection in the wild.
- Designed to ensure only the fittest genetic individuals make it to adulthood. In other words, they aren't all supposed to survive. That's the way mother nature intended it to be, and that's why they lay so many eggs.
- By bringing the eggs all in and 'protecting' them, it ensures that ALL of them do survive, even the runts and genetically inferior ones.
- By sidestepping natural selection in the wild, rearing may well end up watering down the gene pool.

http://www.monarchscience.org/

Adaptation in the lab

Response to artificial selection

- Most traits will response to artificial selection (their means are changed)
- Rate of change per generation (R) in the mean is given by Lush's Breeder's Equation $R = h^2S$
  - S = within generation change in the mean (the strength of selection)
  - h^2= the heritability of the trait (typically between 0.05 and 0.5)

Genetic Concerns

Species bred in captivity often adapt to their captive settings in just a few generations. Frankham (2008) suggests that these genetic adaptations are overwhelmingly deleterious when individuals that have resulted from multiple generations of captive breeding are returned to wild environments. Thus, we do not recommend captive breeding for release as a means to supplement the natural monarch population."

http://monarchjointventure.org/images/uploads/documents/Monarch_Rearing_Instructions.pdf

Concerns about Mass-rearing and Selling of Monarchs By Sonia Altizer, Lincoln Brower, Elizabeth Howard, and Karen Oberhauser

3. Deleterious genetic adaptations Studies in species as different as fruit flies and fish show that animals can adapt to captive conditions in as short as one or two generations. When this happens, researchers see a high frequency of alleles that would be harmful or have reduced survivorship in the wild. The more captive generations, the more extreme this effect. Here is a quote from a review paper on this topic:

"In captivity, species adapt genetically to the captive environment and these genetic adaptations are overwhelmingly deleterious when populations are returned to wild environments" (Frankham 2008).

For all of these reasons, we do not advocate for the release of purchased monarchs to help restore the eastern monarch population. This could do more harm than good, especially if done on a mass-scale.

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Key to response

- Assuming heritable variation, the key to response is strong, focused selection.
- Slow adaption to laboratory conditions can indeed be seen under certain conditions.
- A necessary (but no sufficient) condition is strong selection. This is why most traits respond to focused, strong selection

A much cleaner measure (which requires no assumptions about which traits are under selection nor on the nature of that selection) is I, the **opportunity for selection**, defined as the variance in *relative* fitness:

$$I = \sigma_w^2 = \frac{\sigma_W^2}{\overline{W}^2} \tag{28.5a}$$

This measure was introduced by Crow (1958, reviewed in 1989), who referred to it as the **Index of Total Selection** and was independently developed by O'Donald (1970). I is estimated by

$$\hat{I} = \mathrm{Var}(w) = \frac{n}{n-1}\left(\overline{w^2} - 1\right) \tag{28.5b}$$

Crow noted that if fitness is perfectly heritable ($h^2(\text{fitness}) = 1$), then $I = \Delta\overline{w}$, the scaled change in relative fitness. This follows from the Robertson-Price identity (Equation 6.10), $S = \sigma(z, w)$, with $z = W$ as $R_W = h_W^2 S_W = \sigma(W, w) = \overline{W}\sigma_w^2$, or that $R_W/\overline{W} = \Delta\overline{w} = \sigma_w^2 = I$. Following Arnold and Wade (1984a,b) I is the opportunity for selection, as any variation in individual fitness represents an *opportunity* for a within-generation change in a trait. The opportunity for selection bounds the maximum value of $\bar{\imath}$. This follows by using (respectively), the definition of a correlation ρ, the Price-Robertson identity $S = \sigma(z, w)$, and the fact that $|\rho| \le 1$, to give

$$|\rho_{z,w}| = \frac{|\sigma(z,w)|}{\sigma_z \sigma_w} = \frac{|S|}{\sigma_z\sqrt{I}} \le 1, \tag{28.6a}$$

implying

$$|\bar{\imath}| \le \sqrt{I} \tag{28.6b}$$

$R/\sigma_z = i\, h^2$

Hence, little variation in fitness = little adaptive response

- If most individuals survive, and mating is at random (something a breeder can control), then there is very little selection strength and hence little adaption
  - Cases cited as example of random adaption are those that involve very strong competition and hence very high juvenile mortality (thousands of fly larvae in a small vial, millions of hatchlings in a fishery)
- If mortality is low, then little selection pressure for adaption
- If new stock is constantly being introduced, very little chance of significant adaption

Most response is polygenic

- Let's suppose that a major response has indeed occurred.
- For most traits, this occurs via a so-called polygenic response, resulting from small changes in the allele frequency at a large number of loci, each of small effect
- Hence, the genetic divergence with natural populations would be very small, and random mating would quickly random these gene combinations, essentially remvoing any signal

Dynamics of Allele-Frequency Change

To obtain approximate expressions for the actual dynamics of response we need to follow allele frequency changes over time. Recall from Equation 5.21 that if the character is normally distributed, then $\Delta p \simeq \bar{\imath}\,(\alpha/\sigma_z)\,p$, where p and α are the frequency and average excess of A. This is a weak-selection approximation, as it assumes that $|\bar{\imath}\,\alpha/\sigma_z| \ll 1$. It also assumes that the effects of epistasis, gametic-phase disequilibrium, and genotype × environment interactions are negligible. Assuming random mating, the average effect of an allele equals its average excess and LW Equation 4.15a gives $\alpha = (1-p)a[1+k(1-2p)]$. Substituting yields

$$\Delta p \simeq \frac{a\,\bar{\imath}}{\sigma_z} p(1-p)[1+k(1-2p)] \qquad (25.3)$$

If the gene has a small effect, $a/\sigma_z << 1$, then any Significant change in its frequency requires many (> 50) generations

Frankham's (2008) concern

- A species is very rare in nature, so a captive breeding program produces a laboratory population on the same or larger than the entire natural population.
- Strong selection (due to high juvenile mortality) occurs within the laboratory population, resulting in a response
- These are then released, flooding (i.e. m >> ½) the gene pool

Best practices to avoid adaption

- Moderate population sizes (~ 1000), as this reduces the effect of selection (drift becomes more important)
- Conditions to ensure low mortality
- Continual introduction of native stock (~10 % per generation is a good rough rule)
- Random mating of adults

Evidence?

- "There is clear evidence showing reared monarchs are less fit than wild ones in the migratory generation"
  - Dr. Andy Davis, http://akdavis6.wix.com/monarchscience#!What-everyone-needs-to-know-about-rearing-monarchs-from-a-science-standpoint/cy97/55e3736b0cf28ffc7eec64ac
  - Study was Steffy (2015), who examined recovery rates of wild-tagged and reared-tagged adults

A closer look at the data

- Of 7,277 forewing (FW)-tagged wild monarchs, 10 were recovered in Mexico (0.136%), while of 5061 Hindwing (HW)-tagged, 46 were recovered in Mexico (1.13%, a ten-fold increase).
- Out of 1127 FW-tagged reared monarchs, one was recovered (0.089%), and of 1929 HW-tagged reared monarchs, 1 was also recovered (0.052%)
- Davis lumped all together (reared vs. wild) and found a major difference.
- If this is such a factor, one would expect the forewing recovery rates to be also different by the (roughly) same amount, yet they are statistically identical for wild and reared (wild vs reared FW-tagged, Fisher's exact test p=1.0).
- Hence, the outlier here is the HW-tagged wild, as the FW and HW tagged reared agree, and these agree with the FW tagged wild (HW-reared vs FW wild p = 0.48; FW wild vs HW wild, p = 1.0)

Conclusions

- The genetic risk of reasonably maintained rearing programs to wild monarchs is minuscule at best
- All of the issues raised (inbreeding, environmental effects on fitness, spread of deleterious alleles, rapid adaption to laboratory conditions) are either simply incorrect and naive or else based on very unrealistic assumptions about how most butterfly breeders operate.