

International Butterfly Breeder's Association: www.butterflybreeders.org

Interesting Fact:

In the last 32 years, Insect Lore has shipped out over 8 million Painted Lady larvae; and Monarch Watch Program has shipped 250,000 Monarch larvae in the last 8 years. No damage to local butterfly populations or to the environment has been reported as a result of this livestock being sold, raised and released.

Why it's OK to Release Butterflies:

Expert Answers to Butterfly Concerns

- [1\) Do captive-raised Monarchs migrate?
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1) Do captive-raised Monarchs migrate? Yes!

(Dr. O.R. Taylor, Monarch Watch, University of Kansas)

Yes, this is now well established. Please see the PDF files of the recoveries from 1998 on the Monarch Watch website (www.monarchwatch.org). Under the column for Reared, you will find a modest number of yes's for Monarchs that were recovered in Mexico. We have answered the "Do they migrate?" question in several places. Below is the standard reply version we send.

Do indoor-reared Monarchs migrate? *Yes, they do!* We know that Monarchs begin to migrate when day length decreases in late August. Temperatures and the quality of food plants are decreasing at this time as well. One, or a combination of two or more of these factors, leads to reproductive diapause and migratory behavior in Monarchs. But what about Monarchs that are reared indoors? The lighting, temperature, and food plant conditions for these Monarchs may be significantly different than outdoors. Will these Monarchs still migrate? This question was addressed in last year's Season Summary, but we were not able to pose a conclusive answer. However, this year we know of six Monarchs (SEE "RECOVERIES" ON PAGES 5-7) that were raised indoors, tagged, and recaptured a significant distance away; one of these was recaptured in Mexico. In general, these Monarchs were reared under what are probably common classroom conditions. Temperatures ranged from 60-75, caterpillars were exposed to sunlight from a window or daytime classroom lighting, and food plants were obtained from outdoors. Although we cannot say that Monarchs reared under any conditions will migrate, it appears that some classroom conditions are such that butterflies reared indoors will enter reproductive diapause and will migrate.

2) Do Monarchs that are transported great distances from their natal origin migrate to Michoacan, Mexico? *Yes!*

(Dr. O.R. Taylor, Monarch Watch, University of Kansas)

The prevalent view seems to be that DNA is encoded to produce a system that responds to a set of physical cues in a manner that has the effect of guiding the Monarchs to appropriate overwintering locations. (This said, let's be clear that many Monarchs are blown off course, or don't survive the migration for a variety of reasons.)

3) Does release of non-natal origin butterflies invalidate scientific biogeographic studies? *No!*

(Dr. Bruce Walsh, Associate Professor, Dept. of Ecology and Evolutionary Biology, University of Arizona)

The short answer to the issue of releases confusing studies of local population structure is that this is indeed correct with the older methods of using protein markers (electrophoretic markers) to look at population structure. However, the point is somewhat moot for several reasons.

First, releases are likely to be such a very small proportion of the population as to not likely be sampled in any random sample of the population used to examine local structure.

Second, suppose that indeed a very genetically different strain is released and somehow incorporated into a random sample from the population that is used for looking at population structure. Typically, researchers use genetic markers to reconstruct what amounts to a phylogenetic tree of relationships among individuals (marker genotypes) in the sample. Any distinct individual from the new population will show up as major outliers on the tree, with no connecting individuals. If such a tree is not attempted to be reconstruct, these individuals can give larger F_{st} (a statistical for

population structure) than is indicated by the true population. However, studies failing to attempt to reconstruct the local phylogeny are very poorly done and are unlikely to be published under today's standards.

Third, DNA markers are now the norm. Unlike protein markers, one can use dead museum material in many cases for DNA. Hence, material predating any release is likely available if the research simply looks in local collections. Further, using tightly-linked genetic markers (SNPs, for single nucleotide polymorphisms), it is again straightforward to find those individuals that are very distinct, and again we expect gaps between the local individuals and the released individuals.

In summary, unless the released material makes up a significant fraction of the local breeding population (at least over 1 percent and likely over 5 percent), it is unlikely to be obtained in a random population sample. Even if such distinct genotypes are included, standard methods using DNA markers to look at population substructure can detect such extreme, outliers, and hence these do not compromise the studies.

Please also refer to short article provided at the end of this document, by Dr. Bruce Walsh "Transfers of SNPs and STRs."

4) Does genetic drift and natural selection that occurs in summer Monarchs persist? *No!*

(Dr. Sonia M. Altizer, Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey)

First, I would like to clarify several broad issues related to Monarch population differentiation. Populations may become genetically differentiated as a result of two distinct processes: natural selection and genetic drift. Selection and drift tend to differentiate populations, whereas migration tends to homogenize them. One study (Eanes and Koehn, 1979, using allozyme loci) showed that eastern North American Monarchs are highly genetically variable and local breeding demes become significantly genetically differentiated during the summer generations, but that the entire eastern population becomes effectively mixed during the fall migration.

5) Do Monarchs reaching each colony in Mexico originate from all areas of the northern breeding range? *Yes!*

[Refer to the report that summarizes the collaborative research between Monarch Watch (Dr. O. R. Taylor) and Environment Canada scientists Leonard Wassenaar and Keith Hobson on the natal origins of Monarchs overwintering in Mexico, provided in full on Monarch Watch website: www.monarchwatch.org]

The report states that:

Monarch butterflies recovered in overwintering sites were identifiable by their distinct hydrogen and carbon chemical signals that link them to their general region of natal origin.

Wassenaar and Hobson were able to construct a map for the natal origins of the Monarchs in Mexico by using both isotopic home signals for over 600 Monarchs obtained from 13 overwintering colonies. The Monarchs reaching each colony appear to originate from all areas of the northern breeding range. Two of the colonies appeared to have somewhat higher proportions of butterflies from more northerly parts of the range, but in general, the Monarchs are well mixed when they reach Mexico. Tagging results also show mixing at the overwintering sites, though they are restricted to fewer colonies and individuals.

6) Do captive-raised Monarchs that are released somehow weaken the wild population? *No!*

(Question from: Nigel Venters on dplex-l@raven.cc.ukans, answered by Dr. Bruce Walsh)

Nigel Venters:

Bruce, Thanks very much for your posting. In summary what you are saying is, as breeders always introduce new wild stock into their captive stock every year (because successfully overwintering the species is not often achieved, and/or to promote health within the livestock) that captive stock treated this way with a continual interchange of genes with the wild stock will, when released, make no difference at all to the wild populations?

Dr. Bruce Walsh's reply:

Well, you asked for it. "Domesticated Monarchs are bred in different conditions to wild ones. They will, as a result of the fundamental laws of nature be genetically different from those raised under wild conditions?"

Many domesticated species are "feeble" with respect to their wild counterparts for two (very different) reasons. (1) Inbreeding, which requires many generations and is overturned by a single generation of outcrossing to a large random-mating population, and (2) local adaptation due to artificial and natural selection. For example, domesticated turkeys are very different than their wild counterparts due to extreme selection for meatier birds.

In both cases, if ones breeding stock is only a few generations old, this is really insufficient time for significant inbreeding to occur (unless only a very few adults were used to found the stock) and it is unlikely that local adaptation has evolved. Release of inbred Monarchs into the wild poses little threat, as if they are very feeble, they have little chance of mating. If they do mate, most of the effects of inbreeding depression (across many species) are from the fixation of deleterious recessives (for evidence on this point, see Chapter 10 in "Genetics and Analysis of Quantitative Traits" by Mike Lynch and myself), and hence when they mate with wild Monarchs, the deleterious effects of recessive lethals are completely masked.

If the domesticated stocks tend to show local adaptation to rearing conditions that makes them less fit in the wild, then they too will be at a fitness disadvantage. In order

for them to spread their deleterious genes into the population as a whole, the fraction of the population that they comprise must exceed their decrease in fitness. Hence, to spread genes with a 10 percent decrease in fitness, the domesticated Monarchs must comprise more than 10 percent of the population, and must do so for many, many generations.

Nigel Venters asks:

"What you are saying is with a continual interchange of genes with wild stock will, when released, make no difference at all to the wild populations?"

Dr. Bruce Walsh replies:

Basically, yes. Reared material from colonies that are continually flushed with wild material are extremely unlikely to cause genetic harm to the wild population when released.

7) Do captive-raised butterflies spread diseases and parasites to wild populations? **NO!**

(Dr. Harry Kaya, Insect Pathologist)

The insect pathology literature has many examples of pathogens being introduced purposely or accidentally into insect populations. There have also been purposeful introductions of various pathogens for biological control of insect pests. Viruses, bacteria, fungi, protozoans and nematodes have been released and have become established in insect pest populations with the specific aim of using these pathogens for insect control. However, this approach has also met with numerous failures demonstrating that it is not easy to introduce insect pathogens into field populations.

The introduction of pathogens into field populations by the release of captive-raised butterflies can easily be avoided/prevented. By carefully monitoring the rearing facilities and not bringing other species of insects into this facility, the acquisition of exotic pathogens by the captive-raised butterfly can be avoided. Inspections of laboratory facilities and livestock can be made to verify that both are free of diseases. With vigilant sterilization and housekeeping procedures in place, commercial butterfly breeders can indeed produce disease-free livestock for release to the environment.

What about the release of monarch butterflies infected with the obligate protozoan parasite, OE (*Ophryocystis elektroscirrha*)? In this case, this protozoan parasite is already in the natural monarch populations, and there should be no impact of this protozoan with further introductions. Any selection in the laboratory that would create a more virulent isolate of *Ophryocystis elektroscirrha* would not be favored in the natural monarch population.

To stay in business, commercial butterfly breeders must use adequate sterilization and cleanliness practices. If they weren't doing this, most "sick" livestock will expire in the laboratory or rearing facility before reaching the adult stage. Thus, they would simply be out of business.

(Note: Following Question 8, please refer to two letters prepared by Dr. Kaya, Insect Pathologist, to Swallowtail Farms, a commercial butterfly breeding operation. These letters describe the examination and testing of Swallowtail Farms' lab-raised Monarch larvae, adult butterflies and pupal casing, and the resulting conclusion that the livestock was free of protozoan spores and there was no detection of any other micro organism. These examination results demonstrate that it is indeed possible to produce "clean" butterflies in labs.)

8) Three levels of concern have been raised about the "transfer" (release of Monarchs from one geographic area into another):

1. Flooding the gene pool with the "wrong" genotypes (in terms of migration patterns);
2. release of new diseases; and
3. release of new mobile genetic elements

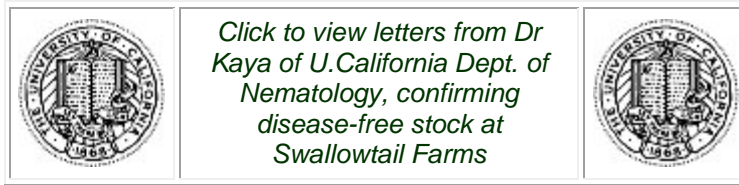
Dr. Bruce Walsh answers:

As to point 1, standard population genetics theory states that the migration rate must be sufficiently large to power a deleterious allele into the population. Introducing an allele (or more generally a genotype) that reduces the fitness of an individual (or its descendants) rarely has any impact, as one must pump in huge numbers (so that the fraction release is a sizable fraction of the total population) just to keep the allele around at low levels. It certainly will not take over the population! Hence, point (1) is not backed up by either good science or good modeling.

Point 2 is easily dealt with, as one would imagine that Monarchs reared in more sterile setting are disease-free (or at least only have the local diseases that they pick up where there are actually raised as larvae). Hence, I see point (2) as being moot, especially in the absence of any data suggesting this is a problem or even a potential problem.

Point 3 is a hypothetical point raised, as the concern is that a genetic virus could be spread from one strain into another. Again, there is no evidence that such viruses exist in Monarchs (some have been found in fruit flies).

Finally, the whole issue of transfer is moot if Monarch from different geographic regions are essentially genetically identical. This is easily testable, and one would simply compute what is called an F_{st} (read as F-ST) value, which measures what fraction of the total variation is due to between-population difference. One could easily use microsatellites or other DNA markers to compare within- and between-population variation to see if there are significant differences between eastern and western Monarch.



Subject: Transfers of SNPs and STRs

(Dr. Bruce Walsh)

Upon reading some of the recent (and past) posts on transfers, I was struck that a major (perhaps the driving) undercurrent is not really biological concern for the population, but rather that such releases would undermine future attempts to look at the historical migration patterns.

As I briefly commented on recently, this need not be the case if some of the more recent molecular marker methods are used. Since most of the list members might not be aware of these, here is a brief introduction.

With our ability to quickly look at the DNA sequence of just about any gene from just about any organism, we can directly score variation at the DNA level. Two types of variation in the sequence of DNA bases have been used as molecular markers - Single nucleotide polymorphisms (SNPs, often pronounced "snips") and Simple tandem arrays (STRs, often pronounced "strips"). SNPs are differences in single base pairs between some individuals in the population at a particular location in the DNA, while STRs are changes in the size of DNA regions showing repeats. For example, GGATATATATCG is a region of DNA with three "AT" repeats, while GGATATATCG has only three repeats. Such simple short repeats tend to mutate at high rates (and hence give different array sizes), while SNPs have far lower mutation rates. Because of these differences in mutation rates, one can use a linked pair of SNP-STR sites to actually gauge the age of this DNA region relative to a standard from which it was drawn. For example, if (say) at a particular SNP, western populations tend to have an "A" allele, while eastern populations have another base (say "C"), when we can estimate the age of the "A" allele in our sample (from its western origin) by looking at the frequencies of linked SNPs.

This allows us to distinguish between very recent migration events (such as might occur via transfers) from historical migration events (those which have occurred at lower frequencies, but at a relatively constant rate over long periods of time). This approach

has been widely used by human population geneticists (such as the Kidds at Yale) to date both recent and historical human migrations.

Subject: Biogeographic Studies

(Dr. Bruce Walsh)

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The Experts

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