### Plant of the Day



*Nymphaea thermarum* is the world's smallest water lily (lily pads about 1cm)

Self compatible

Discovered in 1987

Extinct from the wild (habitat destruction)

Grew in a hot spring in Rwanda (a few square meters of habitat)

Saved by growing from seed at Kew Gardens

## **Big Questions in Conservation Genetics**

What fraction of the world's species are in danger of extinction?

What is the role of genetic factors in extinction?

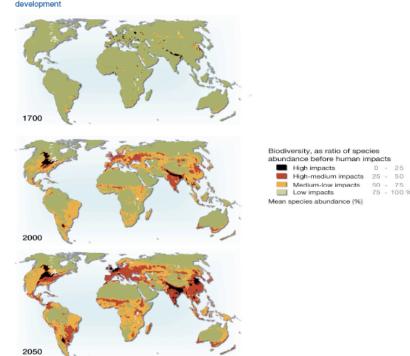
How quickly can genetic factors cause extinction?



"We've worked out what the creature looked like – now all we need to do is find out why it became extinct."

## Biodiversity in trouble: the sixth mass extinction

- Globally, one in five vertebrate and plant species are going extinct Within 100 years 1/2 to 2/3 of all species are will be extinct or endangered
- Current extinction rates are at least 1000 times that of background
- levels



Source: GLOBIO; Alkemade et al., 2009

# What are the major causes of the current biodiversity crisis?

People: the ultimate invasive species!

- -Habitat destruction, degradation and fragmentation
- -Overexploitation
- -Invasive species
- -Climate change

## Why should we care about biodiversity?

- -intrinsic value:
- Moral argument

#### -extrinsic value:

Economic benefits, both direct (goods) and indirect (services) Genetic resources

Aesthetic value and recreation

## Why should we care?

"The future of humanity is inextricably tied to the fate of the natural world. In perpetuating this, the Earth's sixth mass extinction, we may ultimately compromise our own ability to SURVIVE." - Letter to U.S. Senate by E.O. Wilson and 10 other prominent scientists.

# Conservation biology of plant species in Canada

COSEWIC:(Committee on the Status of Endangered Wildlife in Canada) is a committee of experts that assesses and designates which wildlife species are in some danger

#### of disappearing from Canada.

	Mammals / Mammifères	Birds / Oiseaux	Reptiles / Reptiles	Amphibians / Amphibiens	Fishes / Poissons	Arthropods / Arthropodes	Molluscs / Mollusques	Vascular Plants /   Plantes vasculaires	Mosses / Mousses	Jeanne Lichens / Lichens	
Extinct/ Disparue	2	3	0	0	7	0	1	0	1	0	14
Extirpated/ Disparue du pays	3	2	4	1	3	3	2	3	1	0	22
Endangered/ En voie de disparition	n 20	29	17	9	46	25	18	94	8	4	270
Threatened/ Menacée	17	24	11	4	33	6	3	49	3	3	153
Special Concern/ Préoccupante	27	20	9	7	49	5	6	39	4	6	172
Total / Totaux	69	78	41	21	138	39	30	185	17	13	631

\*There are now 617 wildlife species in COSEWIC risk categories which include Extirpated, Endangered, Threatened, or Special Concern / II y a maintenant 617 espèces sauvages dans les catégories de risque du COSEPAC qui incluent les catégories << disparue du pays >>, << en voie de disparition >>, << menacée >> et << préoccupante >>.

## Is this all of the endangered plant species in Canada?

2	VIRIDIMONTANUM	CAREX AGGREGATA	OBOVATA	ROBINSONI	NAJAS MARINA	ESCHSCHOLTZI	URTICIFOLIA VAR	PALLIDUM	SSP DEPRESSA	ENEMION SAVILEI	JUNCUS SUBTILIS	ARCTICA	PUSILLUS SSP	HENDERSONI
	AESCULUS GLABRA		DEPAUPERATUM			GASPENSIS	LACKSCHEWITZI		SCAMMANIANA		KOBRESIA SIBIRICA		SUBSIBIRICUS	SILENE REPENS
4	AGALINIS MARTINA	COREX ASSULLATA	LEIBERG VAR 2	SPH/EROCARPUM	ATTENLIAT	PENSYLVANICA	ALICALUS SSP	SPATHULATUM	TUBEROSA	HORNEMANNI SSP	KRIGIA BIRLORA	CAMPEETRIS VAR	BIFLORA	TAIMYRENSIS
5	NEOSCOTICA	VAR AL NECTENS	NER ON ALC		CLAND .	SAME AN WO			WAS INSTONATION	LIND WALK	LONG MALE	CAN & TRIS VAR	DIVERSIFOLIA VAR	PERFOLIATUM
6	PURPUREA	CAF EX BAULEYI		<b>HO</b> N		PARTICIPACIES N	OREALLA DW	OBLO IGIR LI VAR	ONE AFCULM	OREGINENSE	64 970 6A	HUDDELSONI	DIVERSIFOLIA VAR	SEPTENTRIONALE
7	SCROPHULARWOLL	CAREX CRUS-CORM	ALASIONA			THOGLE MILERATIN	ALLIUM VALUUM			HIPEABOALUS	ARCHCA WAY	LAGOPUS VAR	DRUMMONDII	OVALIS
8	ALTISSIMA	VAR COLLECTANEA			IMBRICATUS	NERVOSA		DESCHAMPSIOIDES		LACKSCHEWITZI	INTEGRIFOLIUM	SCAMMANIANA	ELEGANS	SMILAX ILLINOENSIS
9	HYEMALIS	FESTUCACEA	ELEGANS				ACANTHICAR9A		ODONTORHIZA		LEWISIA TRIPHYLLA		SAGITTATA	VAR GILLMANI
10	ALCHEMILLA ALCINA	C ACCOSCERIA	DOMAN GIA LAETZ	ISOSTES HOMELLIL			FERNALDII	CAMPSIS RADICANS		LEIBERGII	LIATRIS ASPERA	CYMBALARIA	CUNEIFOLIA VAR	VAR ONTARIOENSIS
11	GLOMERULAIS	CALE GRIVDA	VAR CIVEREA	LINE IS BIF OF US		HYDROPHILUS			HOOKERI		CALDERI	MORESBIENSIS	AMBIGUA	VAR RACEMOSA
12	RETRORSA			ESACHYCAPUS	AFLIANUM		CHAMAEJASME SSP		OCHOTENSE	POLIOSPERMUS	VERTICILLATUM	PANICUM RIGIDULUA	ARCTICA	ANDROCLADUM
	CORYMBOSA	CAREX LAXA	DRABA MURRAYI		MCCONNELLI	SENVA HEBECARPA			OCHOTENSE VAR	PARKERI	LILAEA SCILLOIDES		DESCHAMPSIOIDES	
14		LEAVENWORTHI CAREX LONGII	DRABA NORVEGICA VAR SORNBORGERI		CANADENSIS FASTIGIATA	HERMAPHRODITA OREGANA VAR	MULTICEPS ANEMONE PIPERI		FLAVULA VAR CONNATA	CERNUUM PYROLIFOLIUM VAR	LILIUM CANADENSE	ALBOROSEUM PYGMAEUM	DISTANS SSP LAURENTIANA	OCHROLEUCA VAGINIFLORUS VAR
16	VAR HIRSUTA		DRABA OGILVIENSIS		PARRYA NUDICAULIS		AROMATICA		BRAINERDI	SPLENDENS	SEPTENTRIONALIS	RADICATUM SSP	VERTICILLATUM	ALASKANA
17			DRABA PEASE/		PENCILLATA		EUCOSMA	APPALACHICA	CORUSCA	ARENICOLA VAR	VAR ANAGALLIDEA	PAPAVER WALPOLE		AMERICANA
	ALPINA VAR	OLIGOSPERMA VAR			PHACELIA MOLLIS		ESCHSCHOLTZIANUS		LUMARIA	MONTANUM	LINUM MEDIUM	SETACEUM	PRINCIDES	DIORANOIDES
	DICHOTOMA		DRABA SCOTTERI	LECHEA PULCHELLA		SSP OGILVIENSIS	HYEMALE		OKENNONI	PAUCICAPITATUS	MEDIUM	PALUSTRIS	ALLENI	STELLARIA OBTUSA
		RETROFLEXA	YUKONENSIS		PILOSA			BRUNNESCENS SSP			LINUM VIRGINANUM		CALIFORNICUS	UMBELLATA
	PURPUREA		FRAGRANS VAR			LACINATUM			SUBORBICULATA	GALETORUM	AURICULATA	PELLAEA GASTONY		SSP RICHI
22	SSP GRISCOMI	CAREX TUMULICOLA	ECHINACEA PALLIDA	PROCUMBENS	OPPOSITIFOLIA	FUSCATUM	LYRATA	CAREX FRANKI	MODOCENSIS SSP	MINUTIFLORA	LISTERA AUSTRALIS	SSP OCCIDENTALIS	RATIBIDA PINNATA	SUAEDA MOQUINI
23	GLOBULARIA	VAR VALLICOLA	ATROPURPUREA	VIOLACEA	AVENACEUM	OVALIS VAR OVALIS	ARABIS MURRAYI	CAREX GMELINI	AMBIGUA	PROFUNDA	SSP FLAVA	VIRGINICA SSP	OXYACANTHOIDES	DUMOSUM VAR
24	LACINIATA	VARELATIOR	GENICULATA	MULTIFICA	RGURATUS	VARJUNCEA	ARABIS SHORTII	HELEONASTES SSP	KELSEYANA	SSP ASPERULUM	BRANDEGEE!	ATTENUATUS VAR	OXYACANTHOIDES	HEUCHERIFORMIS
25	HIRTELLA	VAR	QUADRANGULATA	TRITICOIDES	FIGURATUS SSP	VAR CHLOROLEPIS	LONGESPICA	CAREX HOSTIANA	CASCADENSIS	GAURA BIENNIS	POLYCAR9A	GORMANI	TRACYI	REVOLUTUM
26	LANUGINOSA	WILLDENOWI	ARCTICUM		CILIARIS	VAR MONTICOLA	LONGESPICA VAR	INCURVIFORMIS VAR	RLAVESCENS	HUMILE	LUPINUS KUSCHEI	PHACELIA LYALLII	RORIPPA CALYCINA	TRIFOLIATUM
	QUADRIFOLIA		CAPILLARIS		FIMERIOLATA	VICTORINI	LONGESPICA VAR		ARIETINUM	FREMONTII	LUPINUS MINIMUS	SSP LONGIFOLIA	LASIOCOCCUS	LACINATUM VAR
28	VARIEGATA		HYSSOPIFOLIUS		AMMOPHILA		CHAMISSON'S VAR		GUTTATUM	PUBERULENTA	GROENLANDICA	PHYLA LANCEOLATA	RUBUS NIVALIS	MILLERLORUM
29		SSP MINOR	ERIGERON SALISHI				LANCEOLATA		LAURENTIANA	QUINQUEFOLIA	VAR ACADIENSIS	DIDYMOCAR94 VAR		THLASPI ARCTICUM
30	BODINI		STRICTUM SSP			SPLENDENS	ARNICA LONGIFOLIA		PROTRUSA	ROSSII	LUZULA RUFESCENS		RUMEX ALTISSIMUS	
31	CONVALLARIUS VAR		CAPITATUM VAR						GLAREOSUM		LYCOPUS RUBELLUS		PAUCIFOLIUS	DIVERSILOBUM
32	KENTROPHYTA	MUEHLENBERGII	INCONSPICUUM VAR				ALASKANA		CANESCENS	LEPTOSTACHYA	VIRGINICUS MALAXIS PALUDOSA	TENELLUS	SALIX CHAMISSONIS RICHARDSONI	
34	KENTROPHYTA VAR MOROCYSTIS	CANADENSIS	OCCIDENTALIS VAR ALTISSIMUM		DOUGLASII SSP KWAKIUTLI		PURPURASCENS		ROTUNDIFOLIUM	OCCIDENTALIS HIRSUTULA SSP	MARAH OREGANUS		VAR	CONCINNA
35	ATRIPLEX POWELLI		DUBIUM			STREPTOPOIDES	SULLIVANTI		DICENTRA UNIFLORA		SPECTABILIS	CHORISIANA	VAR OVALIFOLIA	GASPENSIS
36	ATRIPLEX TRUNCATA		COMMUTATA	SSP	TRIFOLIATUS	LANCEOLATUM VAR			BOREALE VAR 1	HACKELIA DIFFUSA	MMULUS ALATUS	DILATATA VAR	SALIX SESSILIFOLIA	
37	VIRGINICA	INCANUM	MACROPHYLLA VAR				ADULTERINUM		LEIBERGII VAR 1	VAR DIFFUSA	MMULUS BREWERI		SALIX TWEEDYI	BADIATA
					SUBJUGA	ACAULIS VAR	MURARIA		WILCOXIANUM		ELEGANS	FERNALDIANA	MENZIESII	SUBULATA
39	HETERODOXA	CISTANTHE TWEEDYI				DASYCARPUM VAR				WHITEDII	MACROCARRA	POALAXIFLORA	CANADENSIS VAR	VAR OLIATA
40	BLEPHILIA CILIATA	OGILVIENSIS	GYMNOSPERMOIDE	OREGANUS VAR	CONTRACTA	BARBINODE	LENTIGINOSUS	AMBIGUA SSP	GORMANI	OCODENTALE	YUKONENSIS	POA NERVOSA	SAUSSUREA DENSA	VAREDULIS
41	BLEPHILIA HIRSUTA	SUBTILIS	BREVISSIMA	LUPINUS VALLICOLA	LEMMONI	SUBNUDUM	NUTZOTINENSIS	CUSICKII	LAEVIGATA	AUTUMNALE VAR	CAULESCENS	POA PORSILDII	CESPITOSA SSP	ALTERNIFOLIA
42	STRICTA	STRIATA VAR	HYPERBOREA	SSP APRICUS	MACRA	SUBNUDUM VAR	RACEMOSUS VAR	CASTILLEJA ELMERI	LAEVIGATA VAR	HEPATICA NOBILIS	MONTIA BOSTOCKII	PSEUDOABBREVIAT	NELSONANA SSP	VICIA CAROLINIANA
	ACUMINATUM		PUBERULA			NEVADENSIS	SOLEROCARPUS		CORYMBOSA	PUMLUS	MONTIA CHAMISSOI		REDOFSKI	CARLOTTAE
	ASCENDENS		FRAXINUS LATIFOLIA		VERTICILLATUM VAR		UMBELLATUS		DRABA DENSIFOLIA	AMERICANA	MONTIA HOWELLII	MACOUNI	SERPYLLIPOLIA	VIOLA HOWELLII
	BOREALE		FUIRENA PUMILA	TANACETIFOLIA	QUERCUS ILICIFOLIA		ACADIENSIS		LONCHOCARRA VAR		SYLVATICA VAR	CERATOPHYLLUM	SAXIFRAGA SPICATA	
	LINEARE	PERSIMILIS	GALIUM CONCINNUM		LOBBII	CYATHIFERUM	FRANKTONI		LONCHOCARRA VAR	CONCOLOR	TENUIRLORA VAR	BOREALE	STELLARIS	DILATATA
47	PARADOXUM		MULTIFLORUM		MAXIMUM		BAPTISIA TINCTORIA		DRABA MACOUNI		CALIFORNICA	ELEGANS	TAYLORII	SSP LINGUIPOLIA
48	PEDUNCULOSUM PSEUDOPINNATUM	OCCIDENTALIS SSP WATSONI	RACEMOSUM RAMOSISSIMUM	MMULUS DENTATUS SSP PUNCTATA	RIBES MONTIGENUM OXYACANTHOIDES		VIRGINICA		PALANDERIANA DRABA PORSILDII	TENUIPES PROLIFICUM	QUITENSE	VERTICILLATA VAR	SCHIZAEA PUSILLA ANCISTROCHAETUS	SSP VENOSA
		ARISTATUS VAR	GENTIANA CLAUSA		RUBUS GULOSUS		BETULA MICHAUXII		DRABA PORSILDII DRABA RUAXES	SCOULERI SSP	NAJAS GRACILLIMA	BOREALE	SORPUS CLINTONI	VARBREVIS
51	BRAVA PILOSA			ODORATISSIMA SSP		UMBILICATA	DENSIFLORA		STENOPETALA	IDAHOA SCAPIGERA		CAURIANUM SSP	HETEROCHAETUS	WAR BREVIS
52	NARDOSMA	CAROLINIANUM SSP		ODORATISSIMA SSP			GLABELLA		DRABA VENTOSA	IMPATIENS AURELLA		DOUGLASII SSP	ANGUSTIFOLIA	
53	CINNOIDES	CESPITOSA SSP	DETONSA SSP		CALYCINA VAR		ASTEROIDES		ECLIPTA PROSTRATA		CUSPIDATA	DOUGLASII SSP	PARVULA VAR	
		CESPITOSA SSP	GEUM VIRGINIANUM		BOREALIS	GIGANTEA	ASTEROIDES VAR		ELEOCHARIS NITIDA	LINARIJFOLIUS	NEMOROSA	FRANKTONI	OREGANA	
55	MARGINATA	CESPITOSA SSP	GILIA SINUATA	ANDINA	SALIX FUSCESCENS		CAMPESTRE		VAR RACEMOSA		PSAMMOPHILA	POLYGALOIDES SSP		
56	TERRESTRIS	OLIARE	GILIA TENERRIMA	ALIFORMIS		BIFLORA	ORENULATUM		ELODEA BIFOLIATA	JUNCUS CONFUSUS			SENECIO FUSCATUS	
	ANDINA		HIBISCUS LAEVIS		SALIX RAUPI		HESPERIUM		ELYMUS CALDERI	MARGINATUS VAR	CORYMBOSA SSP	IMBRICANS	NEWCOMBEI	
58	BREVIFLORA	SESSILIFOLIUM	LONGIPILUM	MACROSPERMA	SSP	MCROCARRA	MONTANUM	RHOMBOIDEA	LANCEOLATUS SSP	JUNCUS OXYMERIS	OXALIS OREGANA	IMBRICANS SSP	SHELDONENSIS	
59	CONTORTA	DIANTHUS REPENS	MARIANUM	PINNATUM	CESPITOSA SSP	PAUPERCULA VAR	MORMO	CLAYTONIA ARCTICA	ELYMUS SIBIRICUS	JUNCUS SECUNDUS	OXYPOLIS RIGIDIOR	KRUCKEBERGII	ROSTRATA	
													+	

## What are we doing?

#### SARA: Species at risk act (2003)

-prevent Canadian species, subspecies, and distinct populations from becoming extirpated or extinct

-provide for the recovery of endangered or threatened species

-prohibit harming individuals of a listed species and their residence

-encourage the management of other species to prevent them from becoming at risk

## What are we doing?

#### The "success" of SARA

-Listing (449/551 recommended)

- Pink sand verbena
- -Recovery plans/action strategies-few identify critical habitat to be protected
- -only one species has an action strategy (Banff Springs snail, located entirely within a National Park)
- -Habitat protection principally applies to federal land (1% of BC)
- -BC has NO legal protection for endangered species (94% provincial crown land 5% private land)
- The B.C. Wildlife Act prevents the direct killing of wildlife. Of the 1,597 species known to be at risk of extinction in B.C., only 4 are listed under the act

http://www.vancouversun.com/opinion/op-ed/losing+battle+diversity+life/3768751/story.html#ixzz1CTdKIIOX

## What is conservation genetics?

aims to apply genetic methods to the conservation and restoration of biodiversity

Some issues in plant conservation genetics:

1) population size: genetic drift and inbreeding

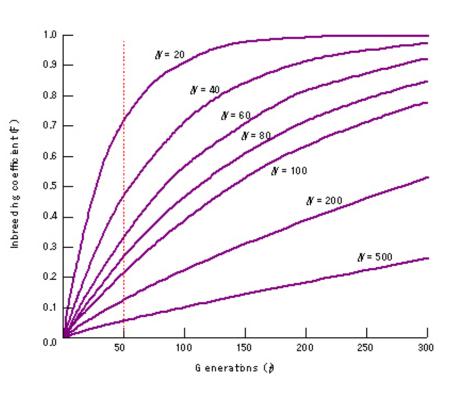
-short term (inbreeding depression)

-long term (effects of genetic diversity and ability to adapt)

2) gene flow: outbreeding depression, genetic assimilation, transgenes

3) units of conservation

# Population size, genetic variation and fitness



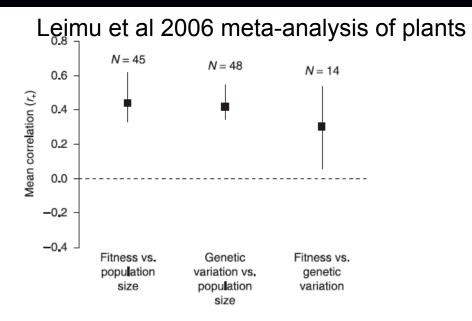


Fig. 1 Mean correlations  $(r_{\star})$  between population size, female fitness and genetic variation. In all figures, bars denote 95% confidence intervals obtained by bootstrapping, and sample size N denotes the number of independent studies included in meta-analysis. The relationships are considered significant if the confidence intervals do not include zero.

Small populations become inbred more rapidly than large populations

Substantial empirical evidence that there is a positive association between population size, genetic variation and fitness

# Population size, genetic variation and fitness

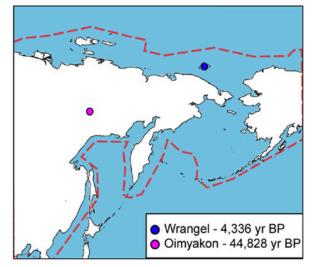
Would you expect a stronger association between population size, genetic variation and fitness in SI or SC species? -SI

#### Why?

-restricted mating opportunities in small SI populations
-inbreeding depression may be weaker in SC species (purging)
-inbreeding maybe high in SC populations irrespective of size

## Genomic signal of inbreeding depression

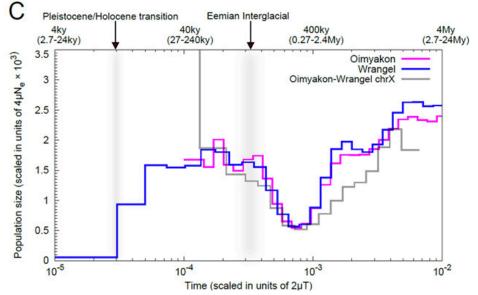
#### Woolly mammoths historical population size from genome <sup>A</sup> <sup>B</sup> data



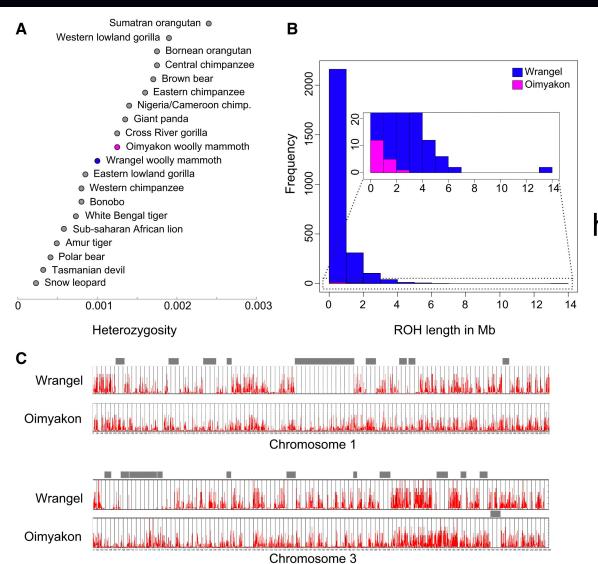
Palkopoulou et al 2015



Sample	<sup>14</sup> C date ±error (years)	Median calibrated date (years)	# raw reads (×10 <sup>6</sup> )	Average coverage	Average read length (bp)	
Wrangel	3,905 ± 47	4,336	1,262	17.1	69	
Oimyakon	41,300 ± 900	44,828	1,401	11.2	55	



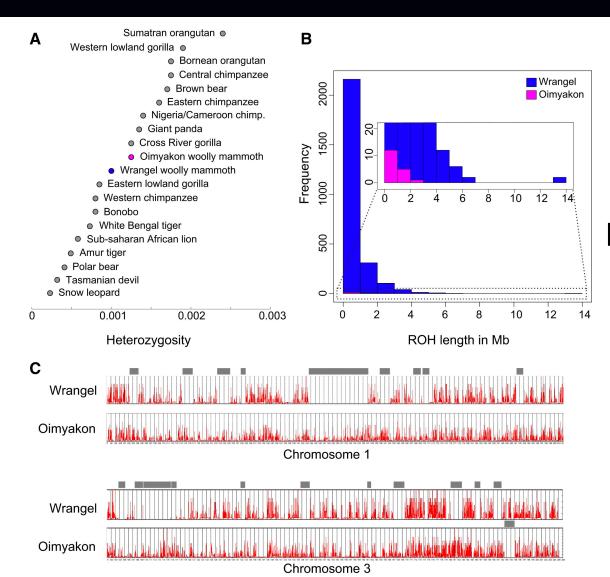
### Genomic signal of inbreeding depression



#### Extended runs of homozygosity (ROH) in inbred mammoth

Red = diversity Grey = ROH

## Genomic signal of inbreeding depression



#### Extended runs of homozygosity (ROH) in inbred mammoth

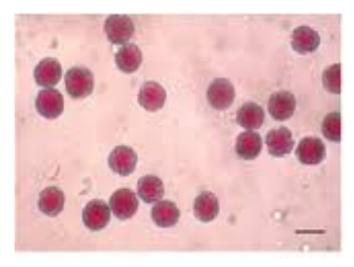


Red = diversity Grey = ROH

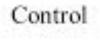
#### Gene Flow: outbreeding depression

<u>Outbreeding Depression</u> – reductions in the fitness of hybrids relative to that of parental individuals

Can result from either intrinsic (hybrid sterility / inviability) or extrinsic (ecological) factors







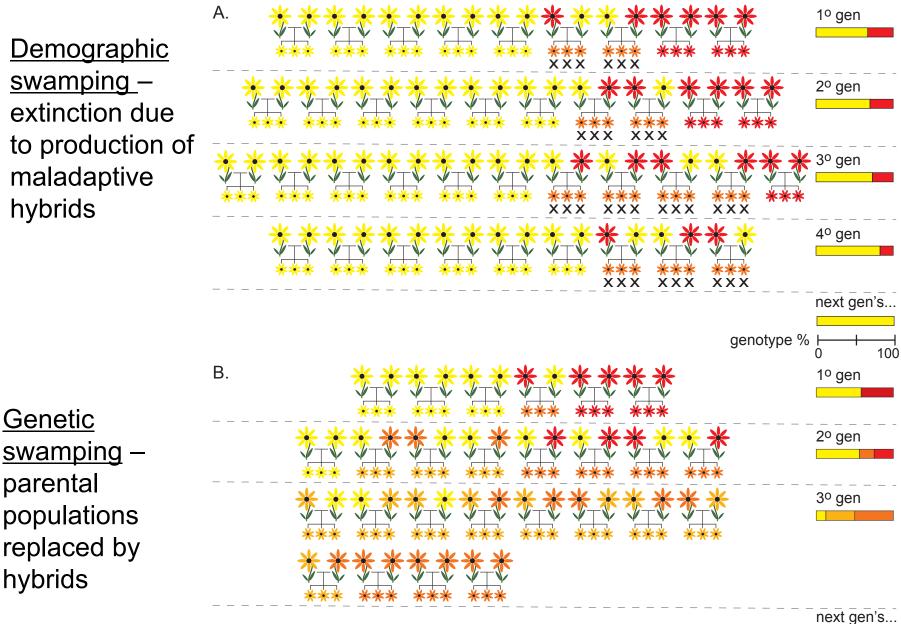


**Demographic** <u>swamping</u> – extinction due to production of maladaptive hybrids

<u>Genetic</u>

parental

hybrids



Todesco et al. 2016

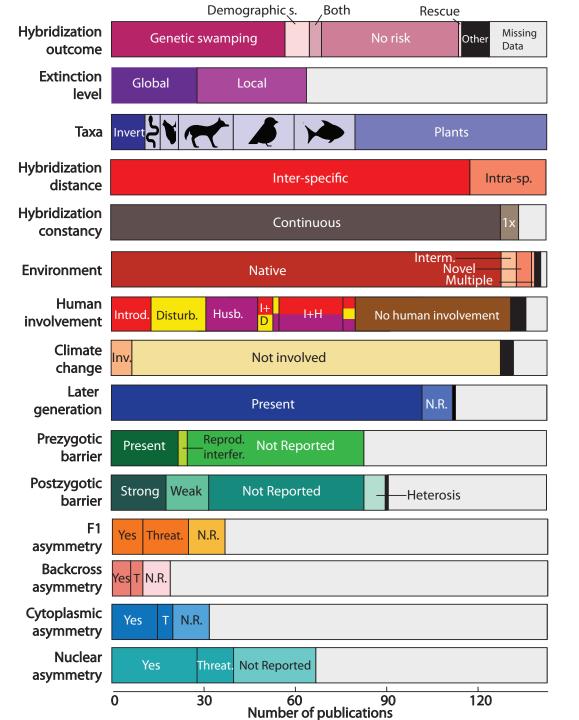
## Example of species at risk through genetic assimilation



Argyranthemum coronopifolium, a rare plant species known from only seven populations in the Canary Islands. Three of the seven populations now contain only hybrids and pure individuals of the invading congener



*Cercocarpus traskiae,* a rare plant species known from only one population on the Santa Cantalina Island. This population now contains only four pure individuals of the species. All others are hybrids (or the more abundant congener). **Figure 2** Overview of results from literature survey of 143 empirical papers (Todesco et al. 2016)



# Gene Flow - the escape of engineered genes

#### **Prevalence of Crop x Wild Hybridization**

#### Gene flow from crop plants into their wild relatives may lead to the escape of engineered genes.

Wheat	Yes	Millet	Yes
Rice	Yes	Common	Yes
		Bean	
Maize	Yes	Rapeseed	Yes
Soybean	Yes	Groundnut	No
Barley	Yes	Sunflower	Yes
Cotton	Yes	Sugar Cane	Yes
Sorghum	Yes		

Gene escape is inevitable for most crops.

Ellstrand et al. (1999)

# The escape of engineered genes

#### Bt protein Cry1Ac toxic to Lepidopteran Insects



Suleima helianthana Sunflower Bud Moth (stem/developing bud) Plagiomimicus spumosum (developing bud; > 50% seed loss)



# The escape of engineered genes

Question:

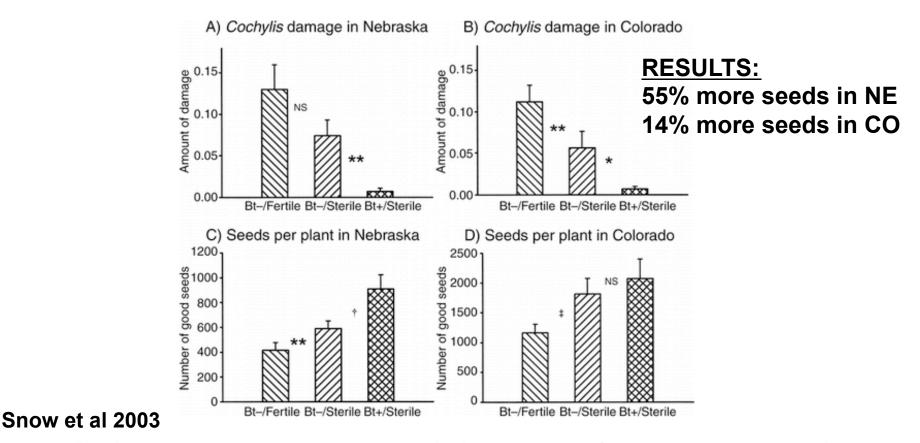
Will the Bt transgene provide an advantage to weedy sunflowers?



#### **Experimental Design**

- backcrossed transgene into wild plants
- planted backcross plants that segregated for transgene at two localities
  compared fitness (fecundity) of plants with or without transgene

## Will the Bt transgene spread into weedy sunflowers?



## Fig. 1. Effects of the Bt transgene and male sterility on relative amounts of sunflower seed damage by *Cochylis* moth species and the number of good seeds (undamaged) per plant in Nebraska and Colorado (USA). Untransformed means and 1 se are shown; N = 58-60 plants in Nebraska, N = 47-49 plants in Colorado. Levels of statistical significance are based on planned contrasts between adjacent treatment means (see Table 1 for details, including methods for reporting damage levels).\*P < 0.05; \*\*P < 0.01; +P = 0.054; +P = 0.077; NS, P > 0.10.



#### **Transgenes: conclusions**

- Bt transgenes are highly advantageous and will spread rapidly into wild sunflower populations
- Why should we care about this?
  - Evolution of weedy sunflowers; threat to specialist insects
- Not all transgenes are beneficial in wild species and thus likely to spread (e.g. resistance to white mold
- Decisions on environmental release should be made on a case-by-case basis.



## Units of Conservation

An **Evolutionarily Significant Unit** (ESU) is a population of organisms that is considered distinct for purposes of conservation.

This term can apply to any species, subspecies, geographic race, or population.

Definitions of an ESU generally include at least one of the following criteria:

- 1) Current geographic separation,
- 2) Genetic differentiation at neutral markers (see below) among related ESUs caused by past restriction of gene flow, or
- 3) Locally adapted phenotypic traits caused by differences in selection.

The equivalent term used by COSEWIC is "Wildlife Species", or for brevity just "species", which is used to refer to biological species, subspecies, varieties, or geographically or genetically distinct populations of organisms.

# Some unanswered questions in conservation genetics/genomics

Does population size reduce the adaptive potential of populations (strong association with neutral markers but will selected loci also be as strongly effected)?

What are the genomic causes of lower fitness in genetically depauperate populations (i.e. genes/pathways are responsible for inbreeding depression)?

How do drift and inbreeding influence plasticity and gene expression?