Studying species: Reproductive isolation

Under the biological species concept: Reproductive isolation is deemed important

- Pre-mating – mate choice
- Post-mating – hybrid sterility, inviability (including ecological effects like mimicry)

Primula vulgaris (primrose, top left), *Primula elatior* (oxslip, top right), and Primula veris (cowslip, left) hybridize & produce occasional intermediates.

Darwin: different species, OK, but many intermediates!

Intermediates rare. Why? Reproductive isolation

Types of reproductive barriers

(“Reproductive isolating mechanisms” cf. Coyne & Orr 2004 p. 28-29)

A) *Pre-mating* {or *pre-zygotic*} *barriers*

a) Ecological/seasonal barriers - mates do not meet

- i) Habitat isolation – “ecogeographic isolation”
- ii) Temporal isolation – allochrony
- iii) Pollinator isolation
- iv) “Immigrant inviability”

b) Behavioral barriers - meet but do not attempt mating

c) Mechanical barriers - attempts at mating do not work!

B) *Post-mating* {also *post-zygotic*} *barriers*

d) Gametic incompatibility – gametes do not fuse due to incompatibilities

(*note: this is* post-mating *but* pre-zygotic)

e) Hybrid inviability – hybrid zygotes have reduced fitness:

- i) genomic factors, selection against hybrids (intrinsic selection)
- ii) hybrids are not suited ecologically (extrinsic selection)
- iii) reduced mating propensity of hybrids (behavioural sterility)

f) Hybrid sterility (even though may survive and mate as normal)

g) Sexual selection against hybrids - disfavoured during mating

As currently formulated[6,7], 'isolating mechanisms' consist of all heritable and environmentally-determined traits that prevent fusion between the populations we call species.

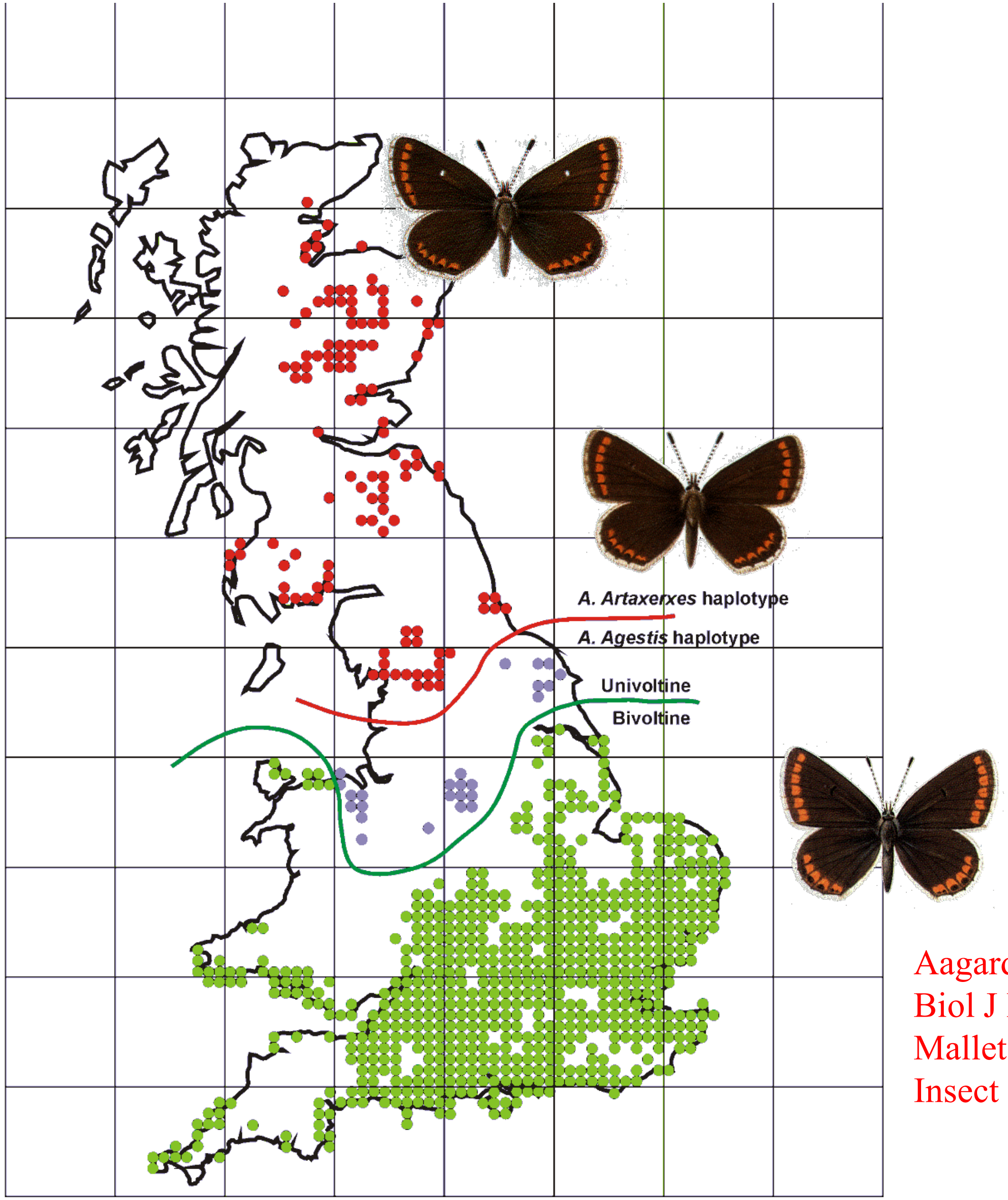
To say that biological species are characterized by 'isolating mechanisms' is therefore an empty statement. To include such an enormous number of different effects under a single label must be one of the most extraordinary pieces of philosophical trickery ever foisted successfully on a community of intelligent human beings.

Mallet 1995 TREE

Aricia agestis / Aricia artaxerxes

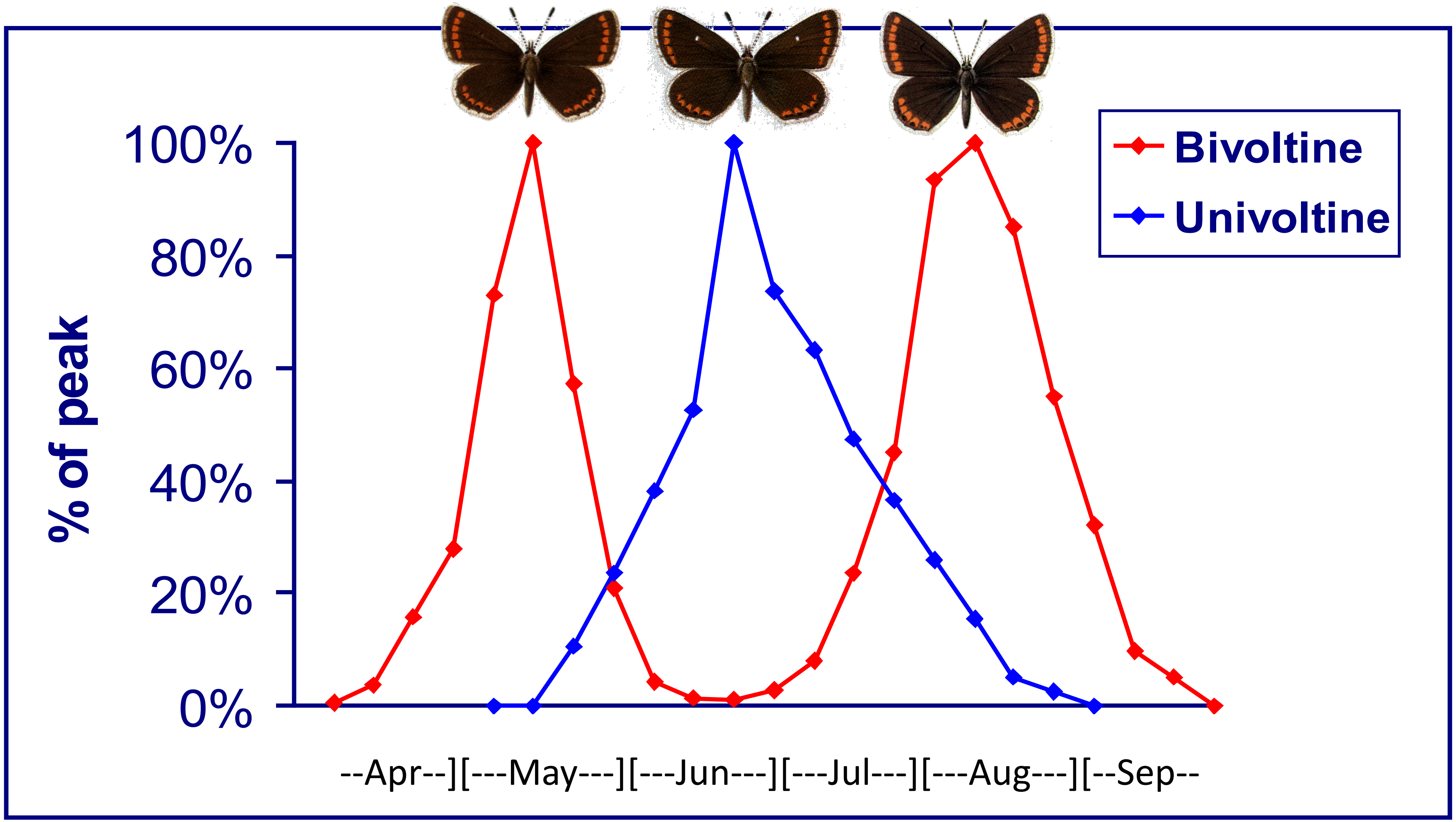
mtDNA studies suggested two species in Britain

N. England & N. Wales *univoltine* (single-brooded) pops. were interpreted as pure *A. agestis* because of mtDNA haplotypes



Aagard K et al. 2002. Biol J Linn Soc
Mallet et al. 2011. Insect Conserv Biodiv

Aricia "agestis" flight periods in N. Wales: allochrony



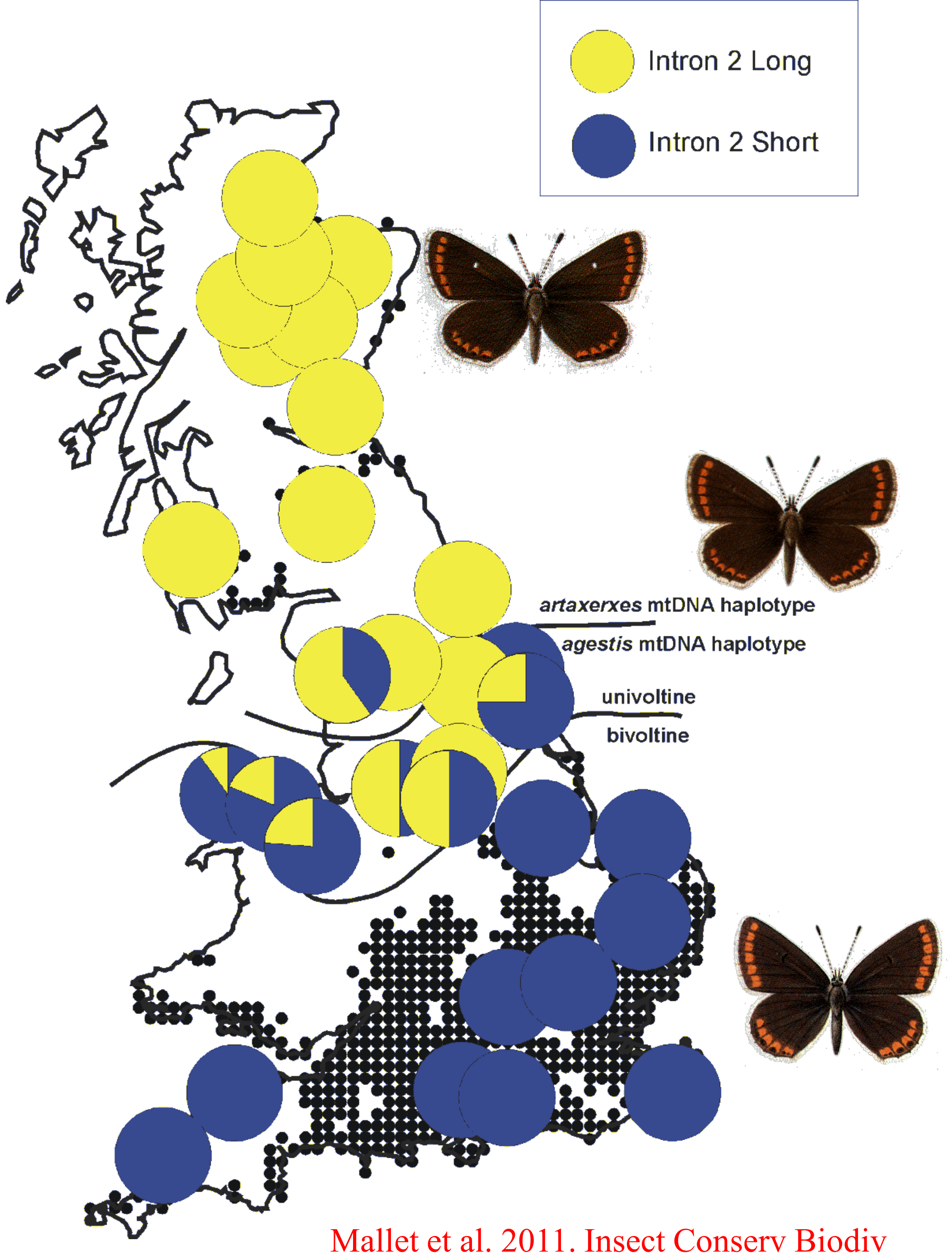
(data from Robert Wilson, U. of Leeds)

But *Aricia* spp. are *BAD* species!

Nuclear gene, *Tpi*

N. English and N. Wales pops. clearly were anciently hybridizing!

Some populations are polymorphic for northern and southern haplotypes



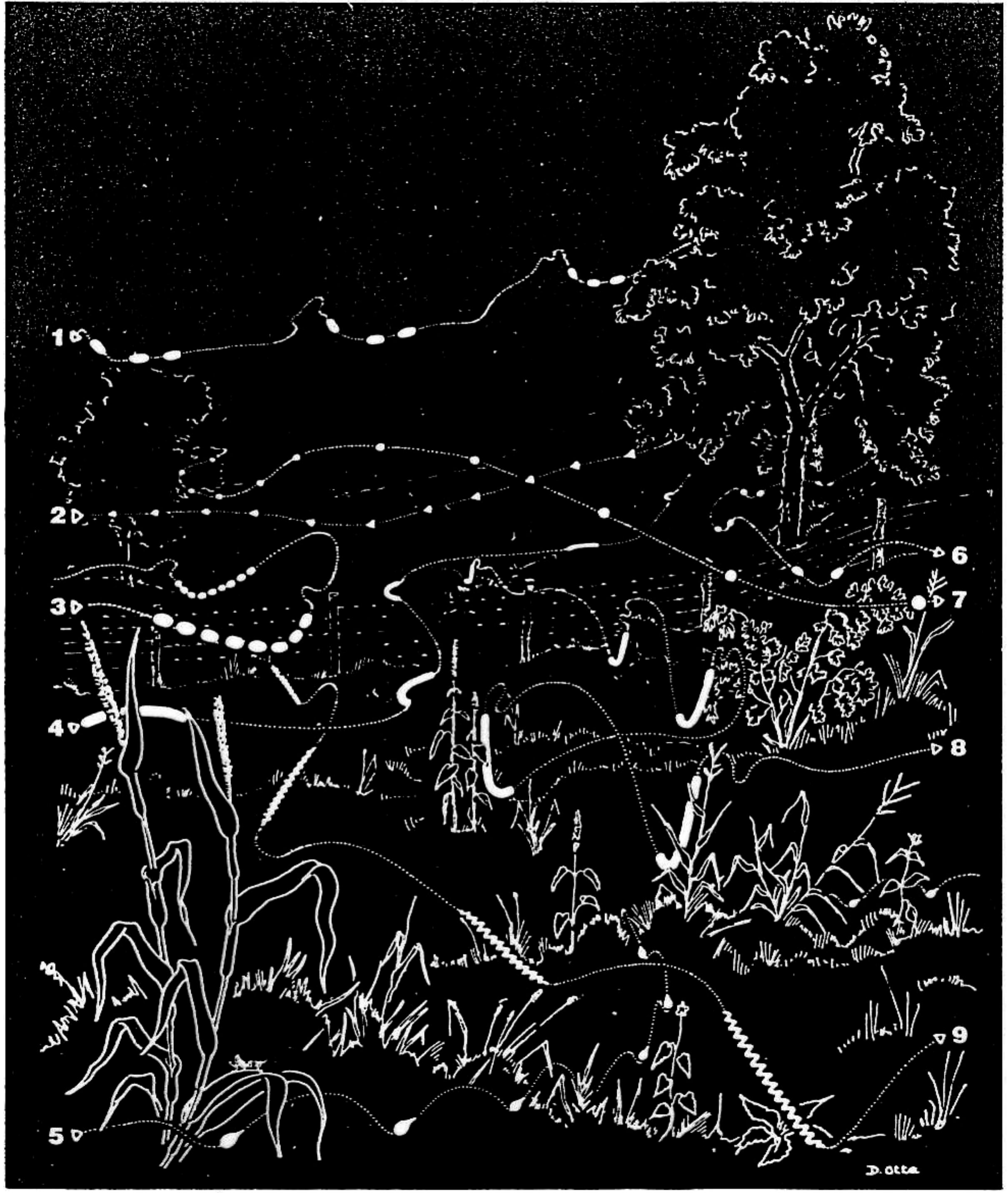
Mallet et al. 2011. Insect Conserv Biodiv

Pre-mating barriers

e.g. Fireflies in
North America

Males "flash" to females in particular codes

Is this "species recognition"?



Pre-mating barriers

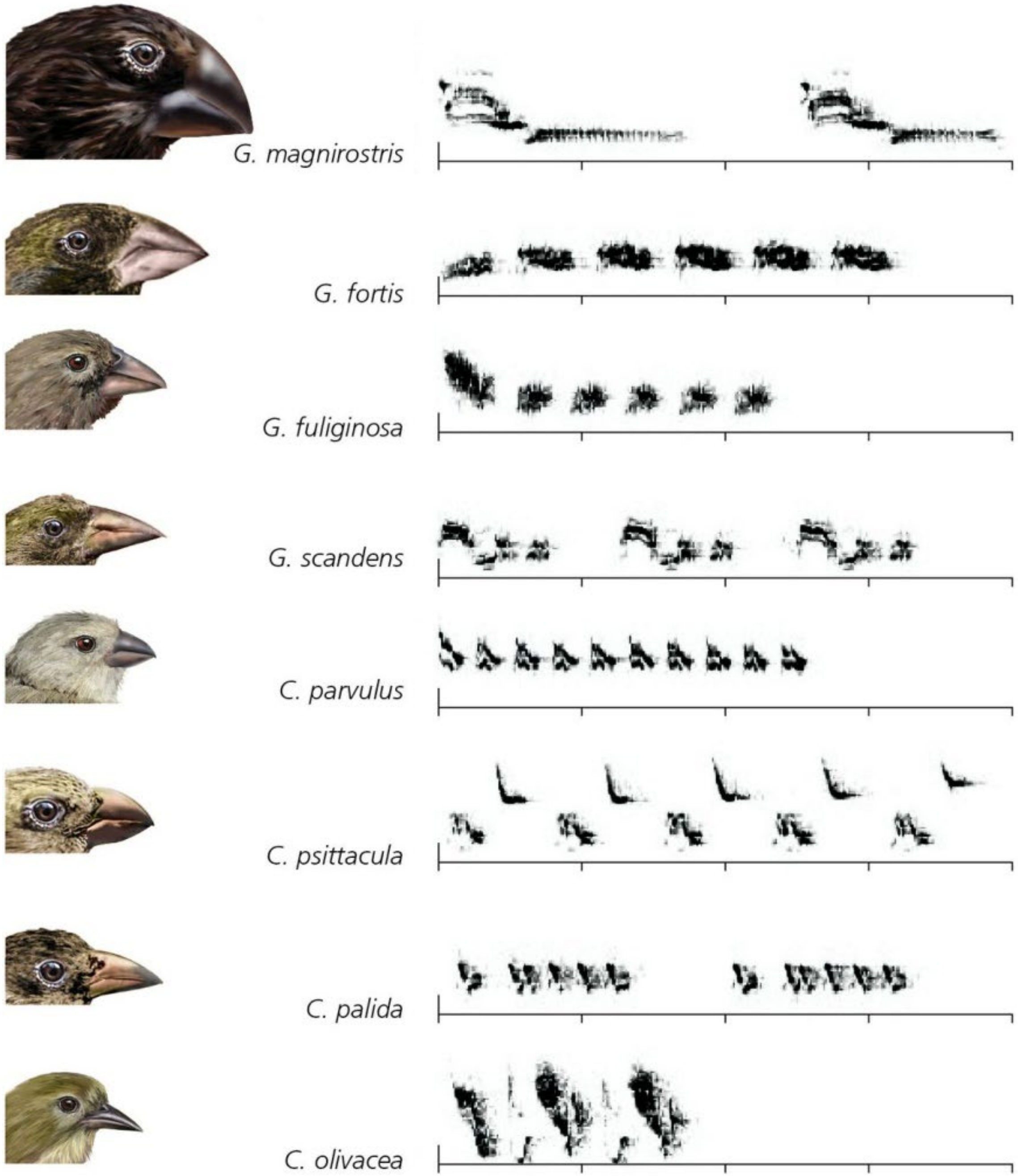
e.g. Darwin's finches on the Galápagos Islands

Beak shape, primarily involved in feeding differences and seed size.

Also affects male call differences as a by-product; calls are used by females in choosing mates

In these Darwin's finches, there is also a learnt component to both male calls and female response to calls; individuals learn from their fathers when nestlings.

When the male dies, females can learn the song of a neighboring male of a different species – causes hybridization.



“Ethological Isolating Mechanisms”

Is their function “species recognition”?

“… it is now realized that many phenomena that have been recorded in the past as furthering intraspecific sexual selection are actually specific recognition marks. Their primary function seems to be to facilitate the meeting and to prevent hybridization between different species.” Mayr 1942

“The functioning of ethological isolating mechanisms ... ‘Species recognition,’ then, is simply the exchange of appropriate stimuli between male and female, which insures the mating of conspecific individuals and prevents hybridization of individuals belonging to separate species.” Mayr 1970

Darwinian view: actually, it’s just about selection for mating between individuals, not whole species. A mixture of sexual and natural selection led to mating patterns. Not really about “species recognition”, although the effect might seem similar.

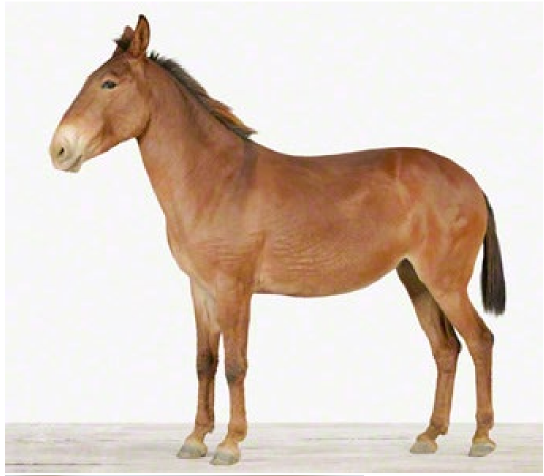
Post-mating barriers

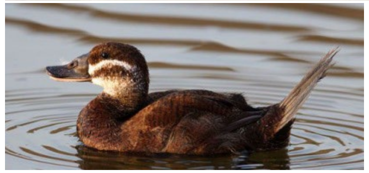
Hybrid inviability and *hybrid sterility* - genomic incompatibility e.g. Mules, chromosomal heterozygotes.

(Some hybrids between species have few sterility or viability problems: e.g. in Darwin's finches, ducks)

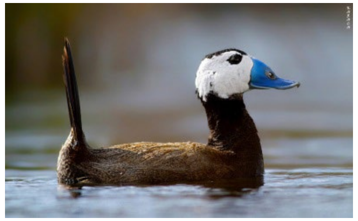
Darwin discussed hybrid sterility at length (Chapter 8, Hybridism). However, he certainly could not "explain" it, at that time. In fact, we're still only beginning to understand how it arises today.

Sterility and inviability are common characteristics of hybrids between species. So some argued Darwin did not explain "The Origin of Species" in his book of that title.





White-headed ♀



White-headed ♂

Ruddy duck ♂

They hybridize!

Drosophila example of post-mating barriers

Drosophila pseudoobscura *Drosophila persimilis*

male

female

They rarely hybridize in nature.
When they do, testes of male hybrids develop poorly. Male hybrids are sterile.

Theodosius Dobzhansky (1937) recognized these forms (then called “Race A” and “Race B”) as separate species. Dobzhansky argued that “reproductive isolation” was the definition of species.

What is [total] reproductive isolation?

“Reproductive isolation (RI) is a core concept in evolutionary biology”

“RI is thus greater than zero when genetic differences between populations reduce the flow of neutral alleles between populations. We show how RI can be quantified in a range of scenarios.”

“After reviewing methods for estimating RI from data, we conclude that it is difficult to measure in practice.”

Westram et al. 2022. J Evol Biol

Reproductive isolation in *Drosophila*:

Comparative evidence

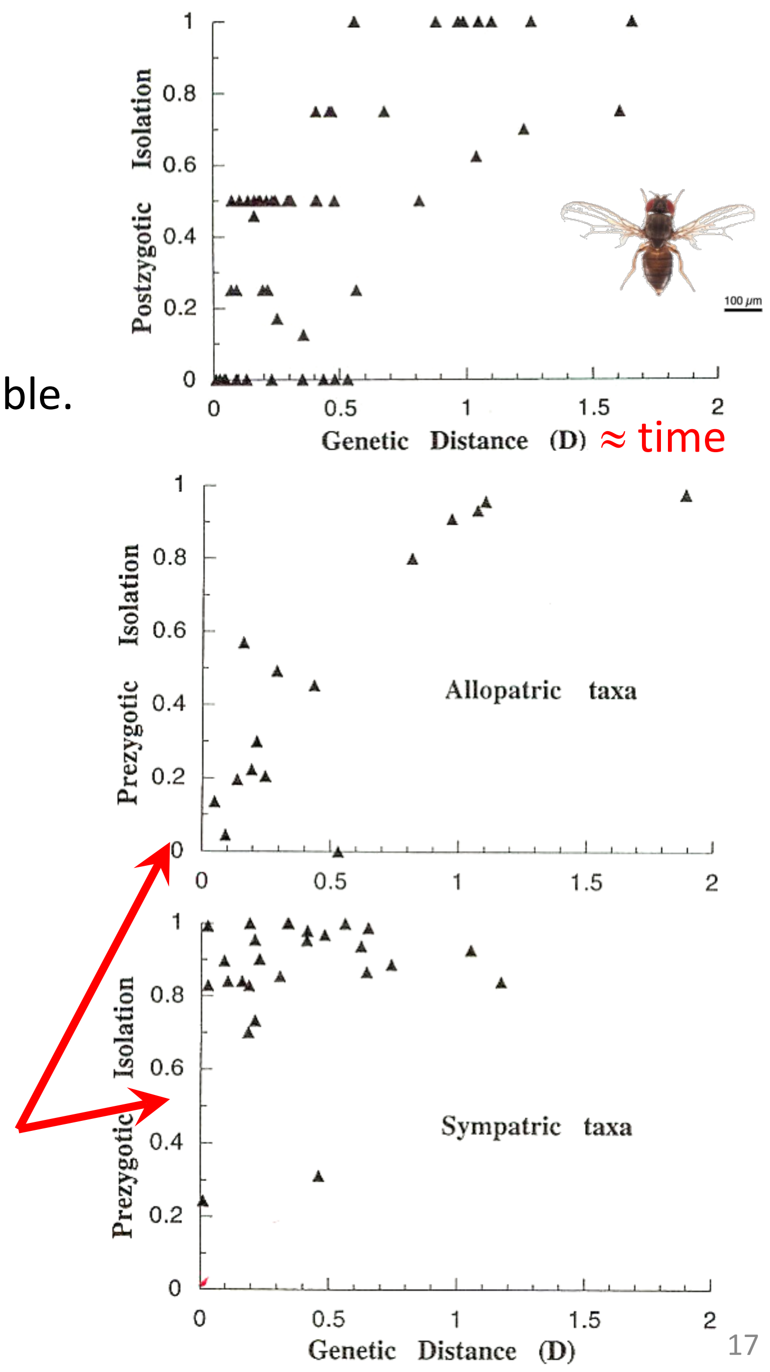
171 pairs of closely related species

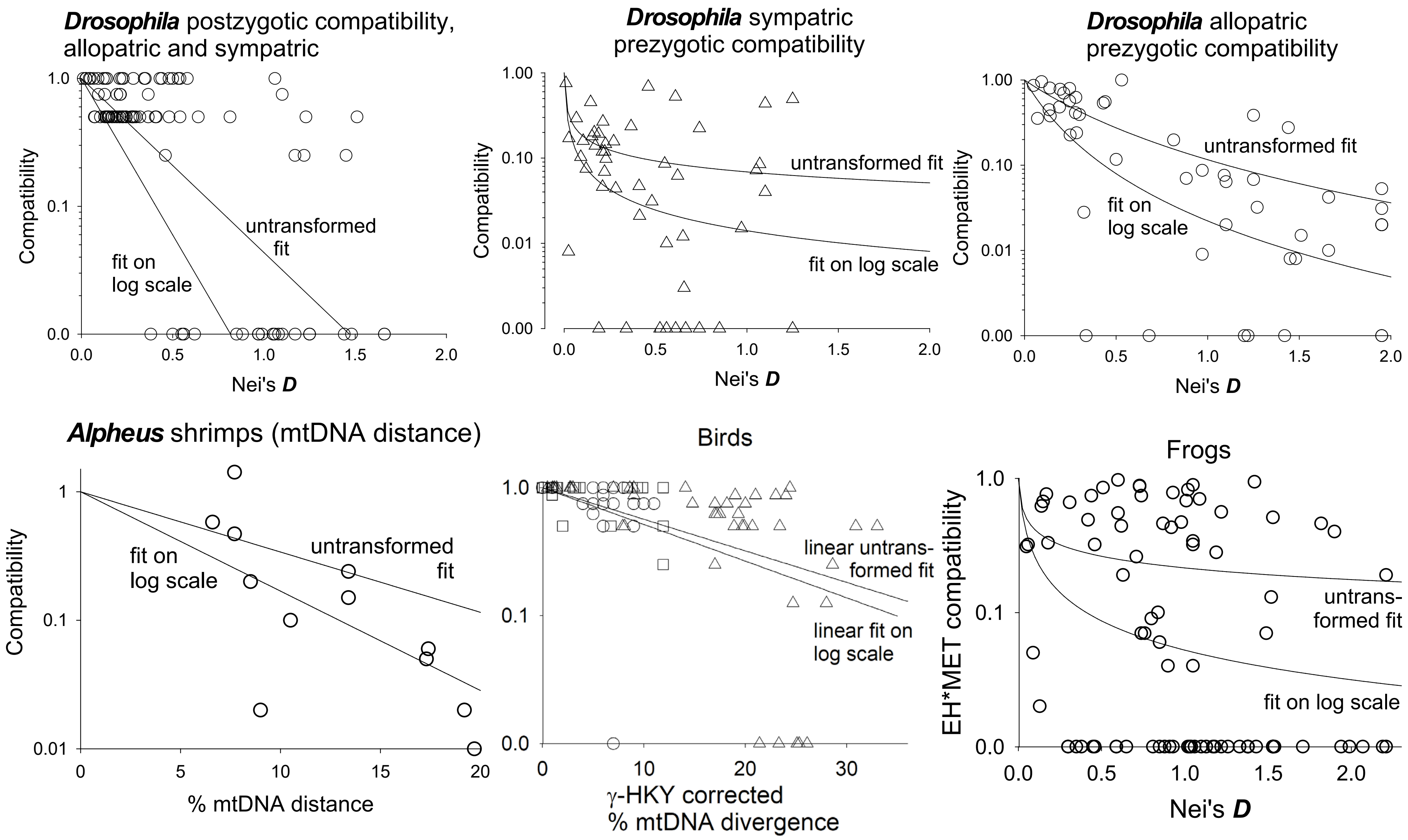
“Post-zygotic isolation” = fraction of crosses in which hybrids are sterile or inviable.
“Pre-zygotic isolation” = fraction of trials of crosses not resulting in mating

Coyne & Orr 1997: Investigated the rate of increase of pre- and post-zygotic isolation with genetic distance (≈ time)

Species accumulate pre-zygotic isolation faster
in sympatry than in allopatry. There was no difference in post-zygotic isolation.

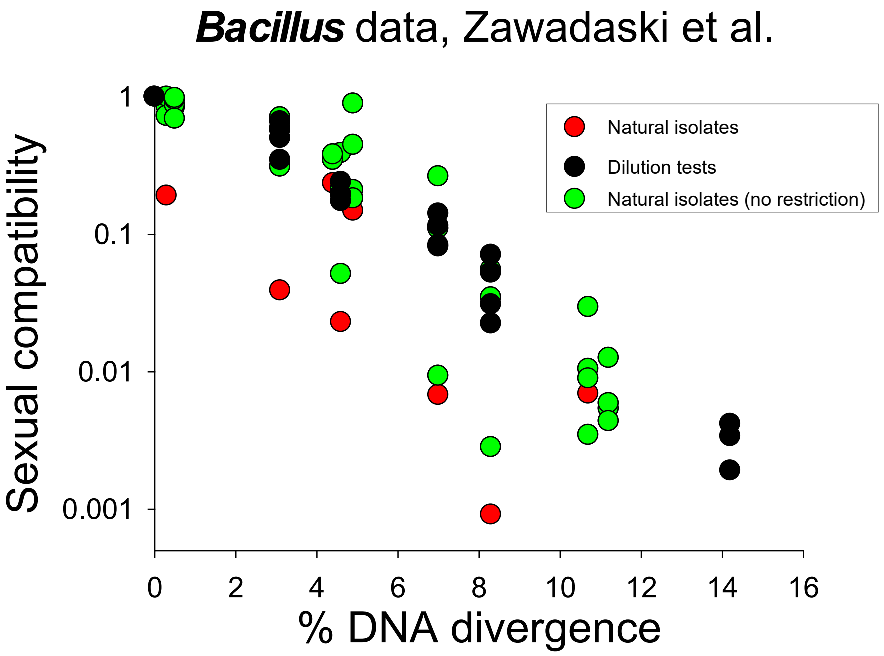
This does suggest something about sympatry of species leads to more rapid evolution of prezygotic isolation.

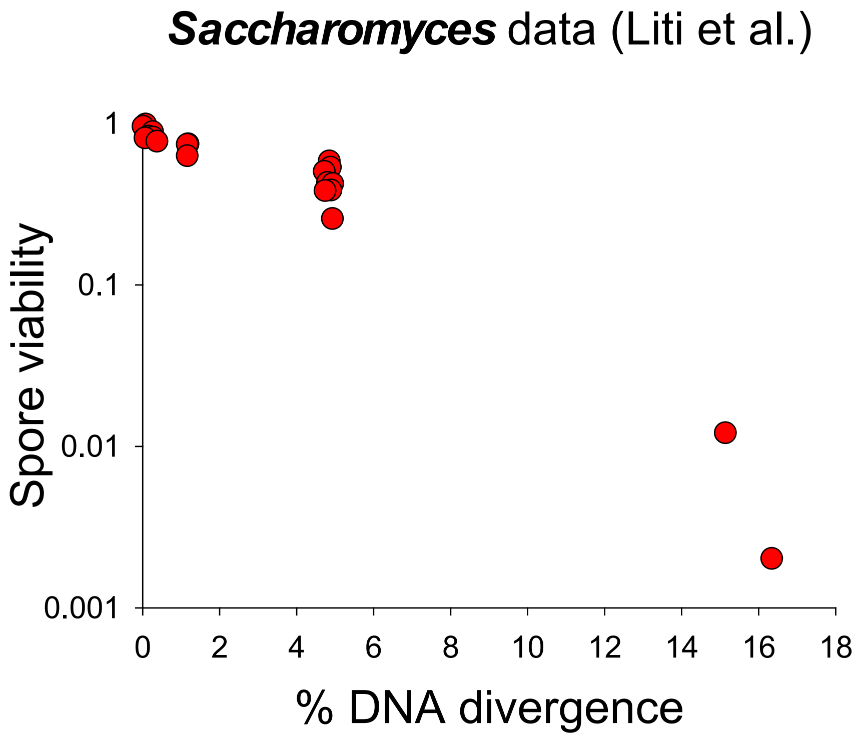




Hybrid sterility/unfitness & prezygotic isolation decline over time; noisy!

Sources : Coyne & Orr, Knowlton et al., Price & Bouvier, Sasa et al. ; from Gourbiere & Mallet, 2010 Evolution.

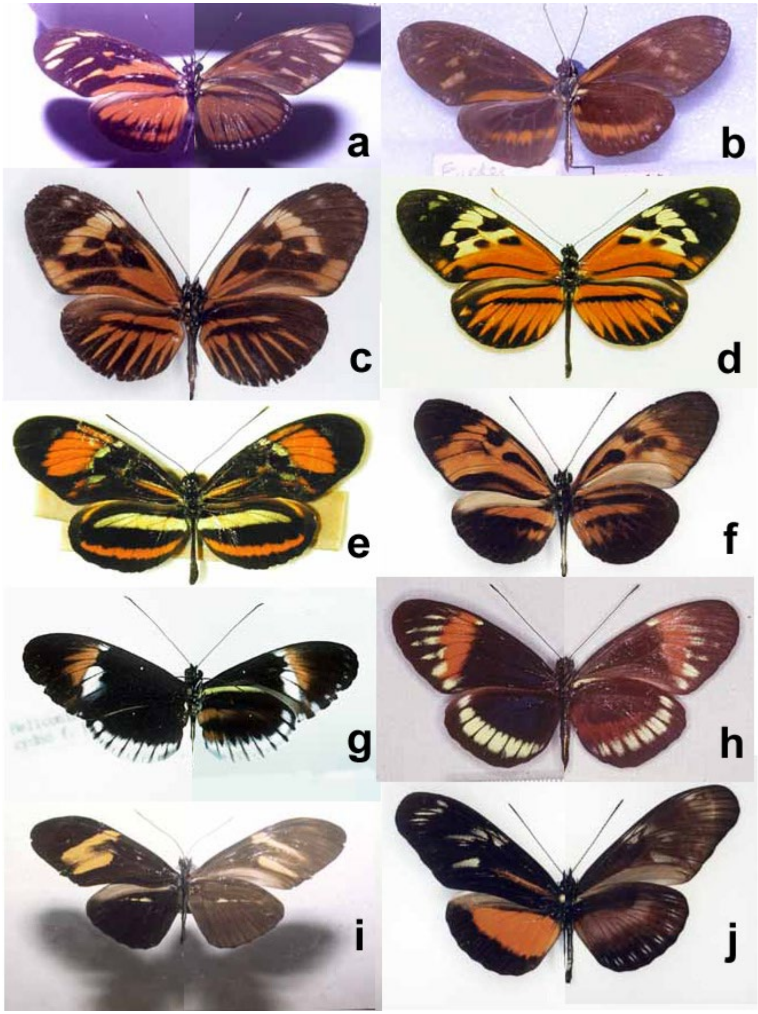


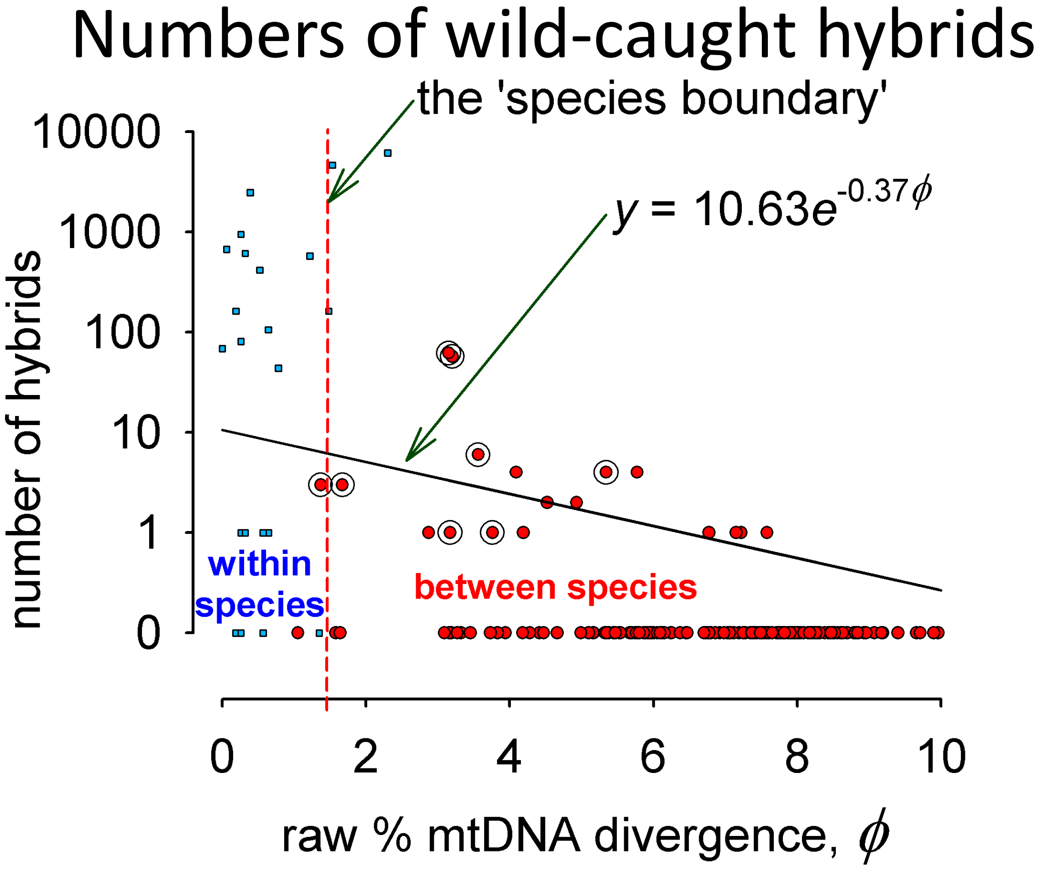


Reproductive isolation – microorganisms give similar, in fact clearer evidence

Sources : Zawadski et al., Liti et al.;
from Gourbiere & Mallet 2010 Evolution

Natural hybridization between *Heliconius* spp: an example of an "exponential failure law"





Mallet, J., et al. 2007. BMC Evolutionary Biology 7:28.

Coyne & Orr 2004 Speciation

"Reproductive isolation" perhaps should measure reduction of gene flow?

If, for example, there are n isolating barriers acting sequentially over the life cycle (I_1 through I_n, where each I, the strength of isolation, falls between 0 and 1), then the total gene flow between a pair of taxa, G, is

$$(1-I_1)(1-I_2)(1-I_3)\ldots(1-I_n), \text{ or } \prod_{i=1}^{n}(1-I_n)$$

Westram et al. 2022. J Evol Biol

Mimulus example

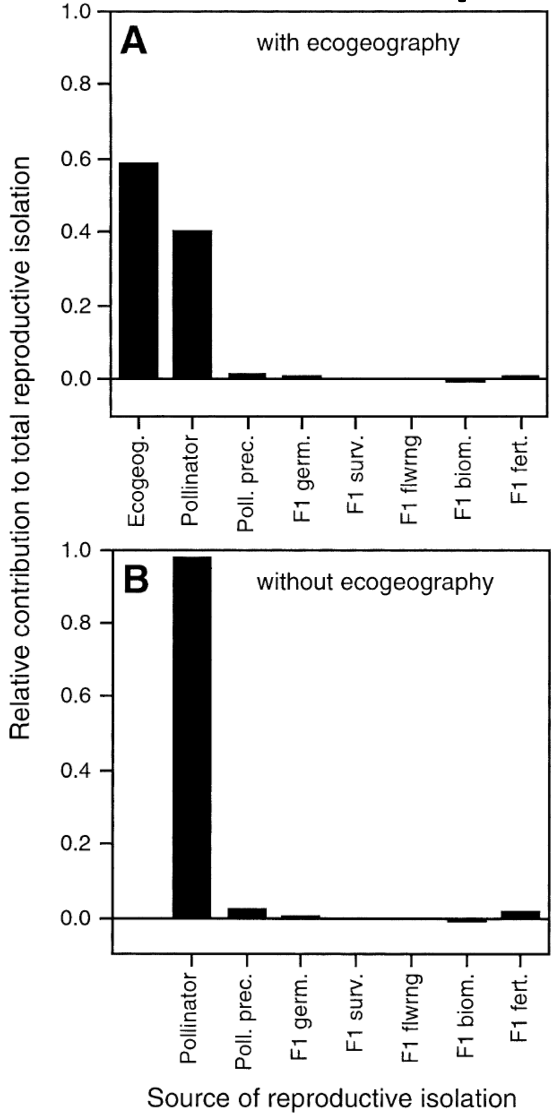
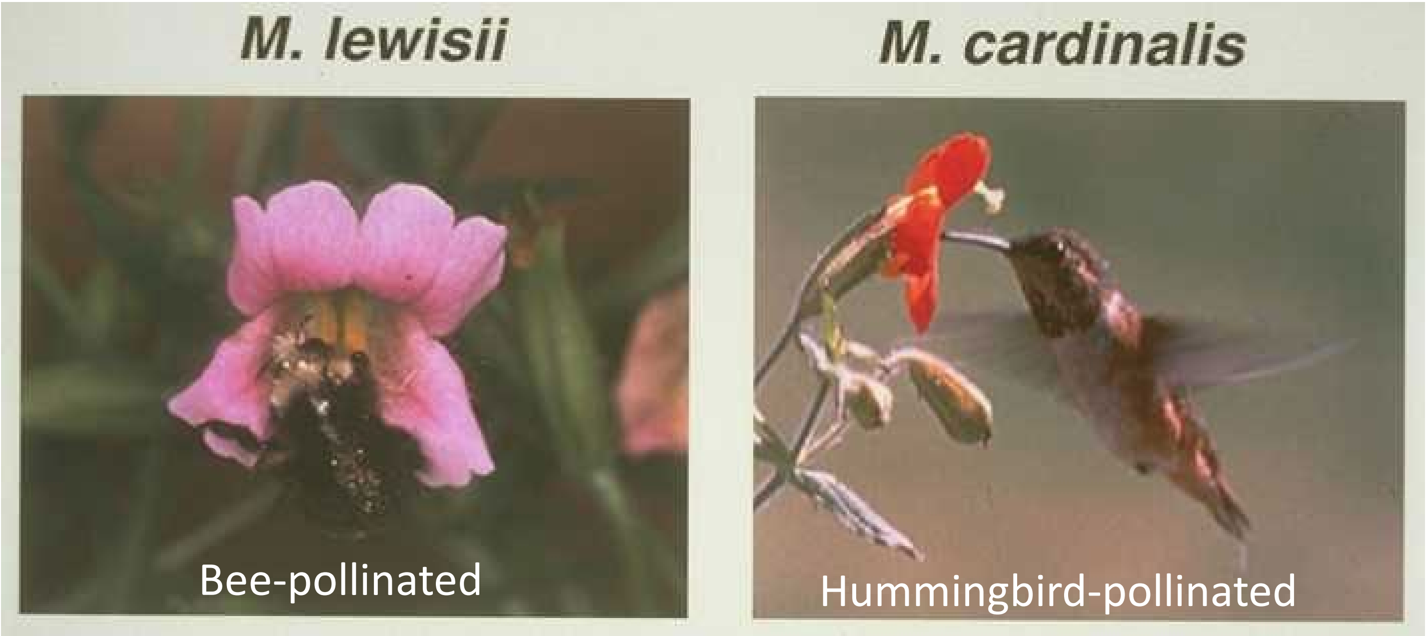
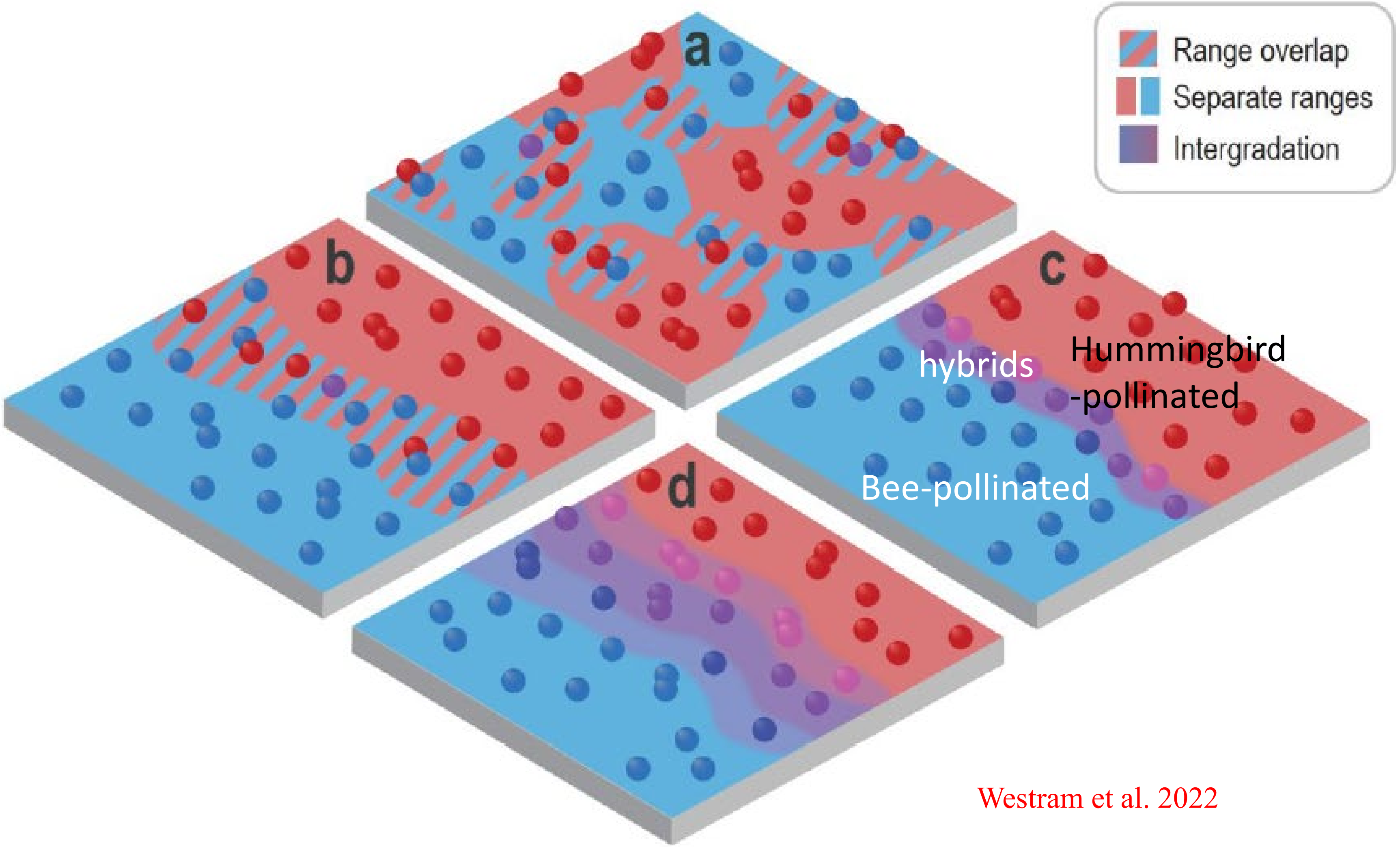


FIG. 4. Relative contributions to total isolation (based on species averages, see Table 2) including (A) all barriers, or (B) for sympatry alone, that is, excluding ecogeographic isolation.



Ramsey et al. 2003. Evolution

Ecogeographic isolation



Westram et al. 2022

Table 2.1 *Reproductive isolating barriers between* Mimulus lewisii *and* M. cardinalis[a]

Isolating barrier	Absolute strength of isolating barrier[b]	Proportional contribution to total isolation
Habitat isolation	0.587	0.587
Pollinator isolation	0.976	0.403
Interspecific pollen competition	0.833	0.0083
F_1 hybrid inviability (seed germination)	0.125	0.0002
F_1 hybrid sterility[c]	0.609	0.0009
Total isolation		0.9986

Source: Ramsey et al. 2003

[a]Figures are averages of values from each of the two species, some recalculated from data of Ramsey et al. Values for the intensity of each isolating barrier range from 0 (interspecific gene flow equal to intraspecific gene flow) to 1 (complete reproductive isolation between species).

[b]Strength of barrier considered in absence of other barriers; 0 = free gene flow, 1 = no gene flow.

[c]Average of male (pollen viability) and female (seed mass) components of sterility.

Ramsey et al. 2003. Evolution
as shown in Coyne & Orr 2004 Speciation book

Conflicting ideas of reproductive isolation

By 2014, there were a large number of conflicting ways of calculating reproductive isolation

Sobel & Chen realized that this was problematic

Needed standardization!

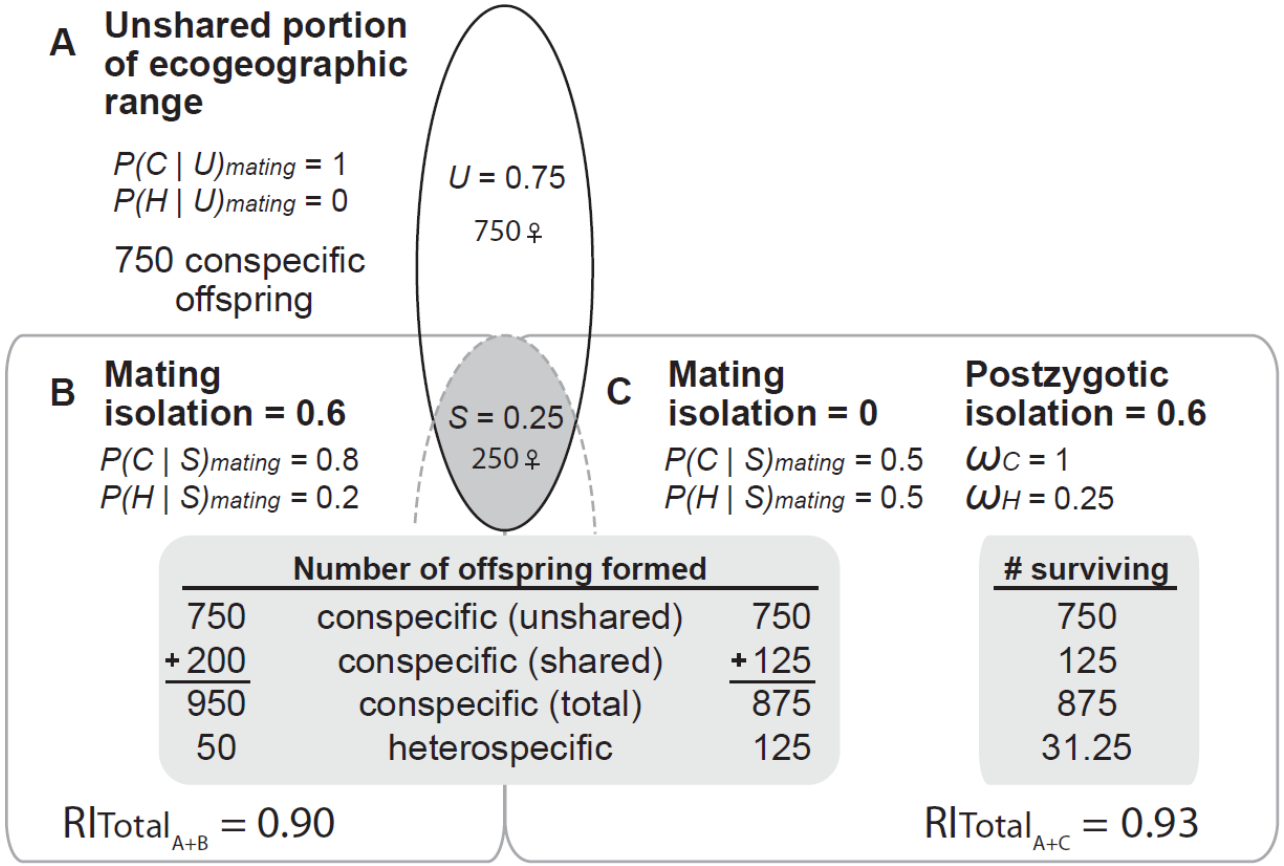
Table 1. Alternative methods for calculating reproductive isolation indexes.

Equation	Type of isolating barriers	Algebraic equivalent	Example reference
$RI = 1 - \frac{\text{frequency of heterospecific matings}}{\text{frequency of homospecific matings}}$	Prezygotic (mating preference)	$RI_1 = 1 - \frac{H}{C}$	Coyne and Orr (1989)
$RI = 1 - \frac{\text{number of cross species foraging bouts}}{\text{total number of foraging bouts}}$	Prezygotic (pollinator fidelity)	$RI_2 = 1 - \frac{H}{H+C}$	Ramsey et al. (2003)
$RI = \frac{\text{\% conspecific females inseminated} - \text{\% alien females inseminated}}{\text{\% conspecific females inseminated} + \text{\% alien females inseminated}}$	Prezygotic (mating preference)	$RI_3 = \frac{C-H}{C+H}$	Stalker (1942)
$RI = 1 - \frac{\text{observed/expected heterospecific matings}}{\text{observed/expected homospecific matings}}$	Prezygotic (flowering time)	$= RI_1$	Martin and Willis (2007)
$RI = 1 - \frac{\text{proportion of hybrid seed}}{(1 - \text{proportion of hybrid seed})}$	Prezygotic (hybridization rate)	$= RI_1$	Brock (2009)
$RI = 1 - \frac{\text{proportion of seeds sired by the minor donor}}{\text{proportion of seeds sired by the major donor}}$	Postpollination (seed production)	$\approx RI_1$	Ruane (2009)
$RI = \frac{(1 - \text{proportion fertile hybrid males/proportion fertile pure males})}{2}$	Postzygotic (hybrid sterility)	$= \frac{1}{2} RI_1$	Bono and Markow (2009)
$RI = \frac{\text{\% of successful allopatric pairings} - \text{\% of successful sympatric pairings}}{\text{\% of successful sympatric pairings}}$	Prezygotic (mating preference)	$= -RI_1$	Hosken et al. (2009)
$RI = 1 - (\text{\% of trials where foreign habitat was chosen})$	Prezygotic (habitat isolation)	$= RI_2$	Nosil et al. (2005)
$RI = \frac{\text{\# of conspecific spawning events} - \text{\# of heterospecific spawning events}}{\text{\# of conspecific spawning events} + \text{\# of heterospecific spawning events}}$	Prezygotic (mating preference)	$= RI_3$	Mendelson (2003)
$RI = 1 - \frac{2 \times \text{average fitness of the hybrid offspring}}{\text{average fitnesses within the two allopatric populations}}$	Postzygotic (incompatibility)	$= RI_3$	Palmer and Feldman (2009)
$RI = 1 - \frac{\text{prob. mating with heterospecific male}}{\text{prob. mating under random choice}(=0.5)}$	Prezygotic (mating preference)	$= RI_3$	Takami et al. (2007)

Sobel & Chen 2014 Evolution

Complexity of existing reproductive isolation measures

$$RI_{4E} = 1 - 2 \times \left(\frac{S_{\text{total}} \times P(H|S) + U_{\text{total}} \times P(H|U)}{S_{\text{total}} \times P(H|S) + U_{\text{total}} \times P(H|U) + S_{\text{total}} \times P(C|S) + U_{\text{total}} \times P(C|U)} \right)$$



Key:

RI_{4E} = 4Eth measure of RI

S = shared geog. distribution

U = unshared geog. Distribution

C = conspecific

H = heterospecific

P = probability of something

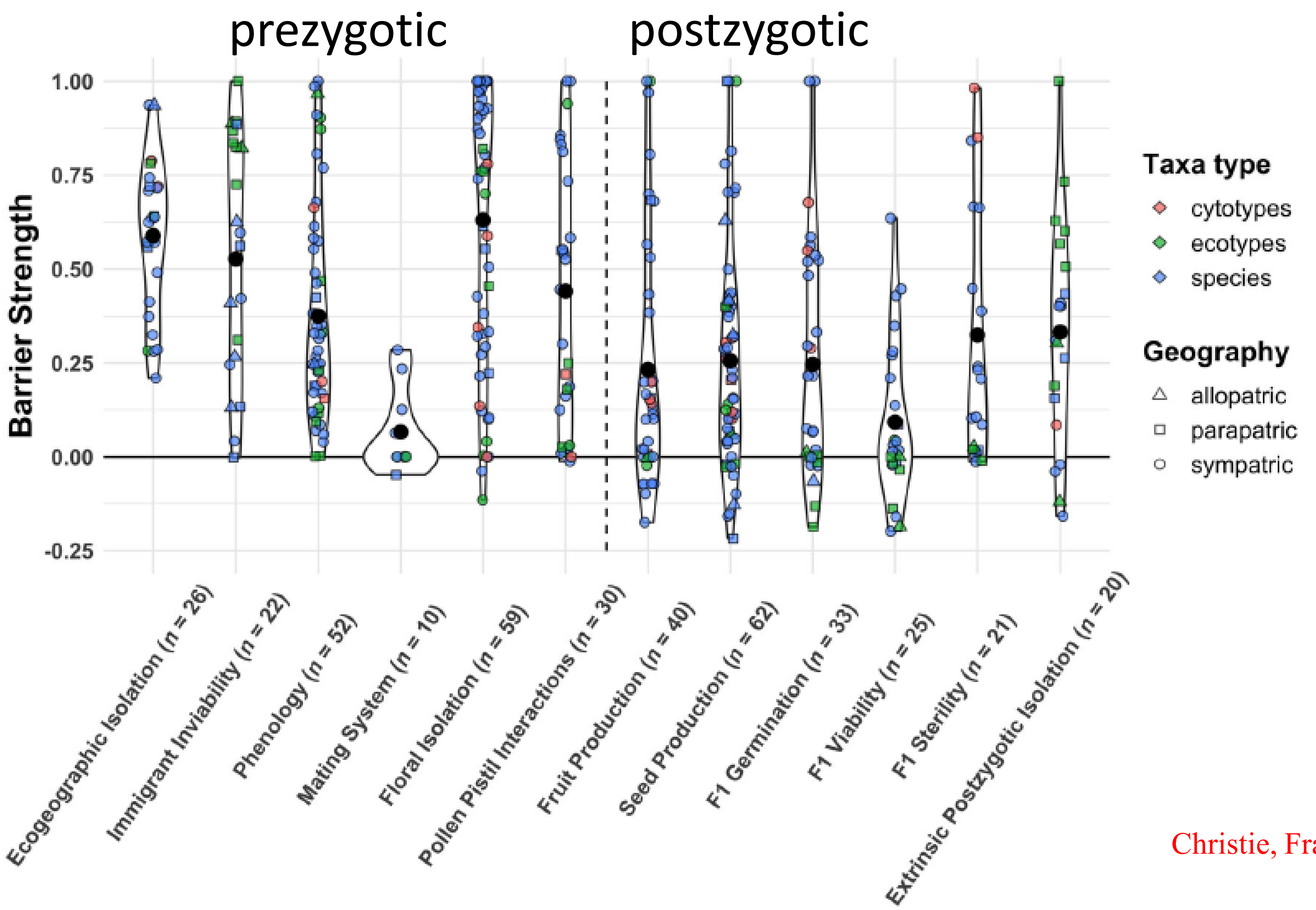
RI = 1 – *P*(gene flow)

P(gene flow) = *H*/(*C*+*H*)

Sobel & Chen 2014 Evolution

Sobel & Chen's 2014 measures of RI as a standard.

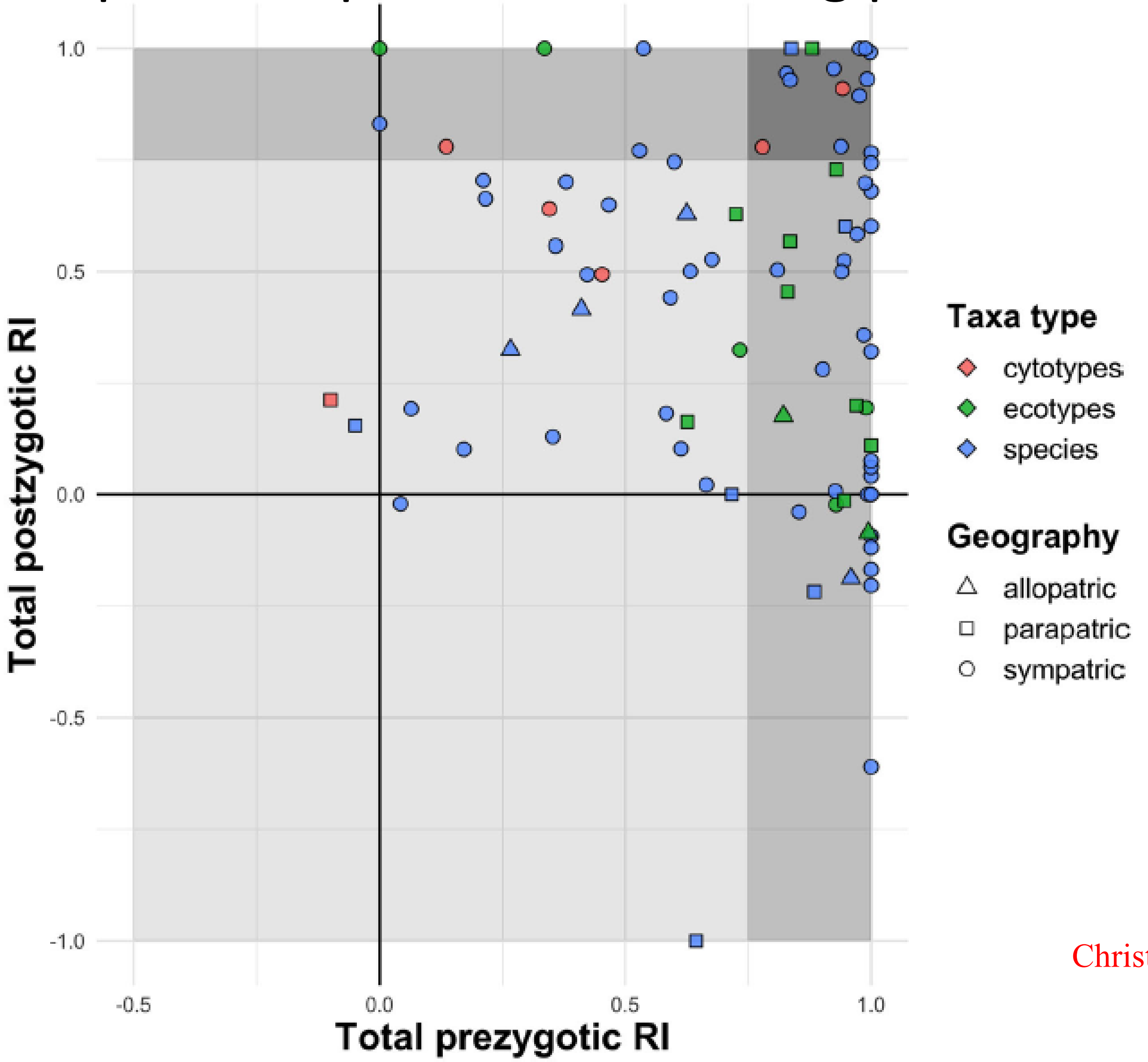
89 pairs of species of flowering plants



Christie, Fraser & Lowry 2022

Is there a correlation between pre- and post- RI?

89 pairs of species of flowering plants



Christie, Fraser & Lowry 2022

Experimental estimation of reproductive isolation

Many studies

Usually only to F1

Yet gene flow may continue past F1, to F2 and backcrosses

Value of RI so measured does not readily predict:

- Existence of separate species
- What is a species
- Long-term gene flow hard to measure
  - & depends on selection vs. neutrality

What is reproductive isolation (RI)?

"Reproductive isolation" should measure reduction of gene flow.

"Following other definitions with a genetic focus, we propose that RI is a quantitative measure of the effect that genetic differences between populations have on gene flow.

"Specifically, RI compares the flow of neutral alleles in the presence of these genetic differences to the flow without any such differences."

"RI is thus greater than zero when genetic differences between populations reduce the flow of neutral alleles between populations."

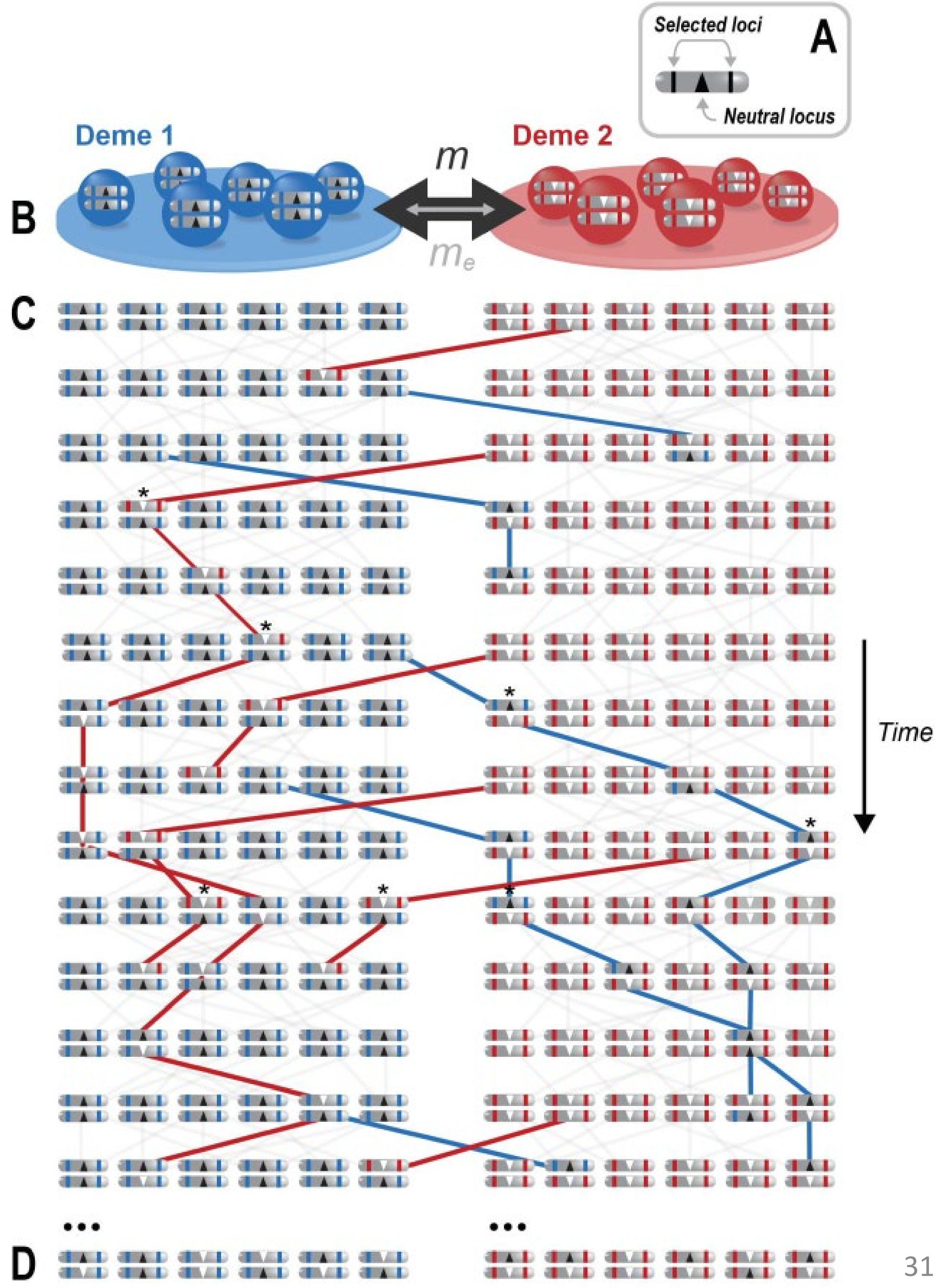
Westram et al. 2022. J Evol Biol

Reproductive isolation
= 1 – (gene flow)
Neutral alleles only.
Over all time!

Westram et al. 2022. J Evol Biol

Selected alleles (coloured)
Neutral alleles (black & white)

Eventually neutral allele frequencies are equalized, but takes much longer with reproductive isolation. Selected alleles act as "barriers"



Reproductive isolation: my opinion, for what it’s worth!

Reproductive isolation is a sort of heuristic that “explains” the separateness of species in some way. Of course sexual species are reproductive isolated! I agree, but what is it?

Reproductive barriers: what do these include? All sources of divergent selection and mate choice? These seem like very different kinds of things!

When we try to critically define, or measure RI, it gets tricky. Do we mean the things that prevent gene flow in the F1? (Coyne & Orr, Sobel & Chen). And maybe backcrosses & F2? Or for all time? (Westram et al.)

There’s a notion that speciation is not “complete” until there is zero gene flow. But plenty of species do hybridize, and it would be hard to insist on complete lack of gene flow.

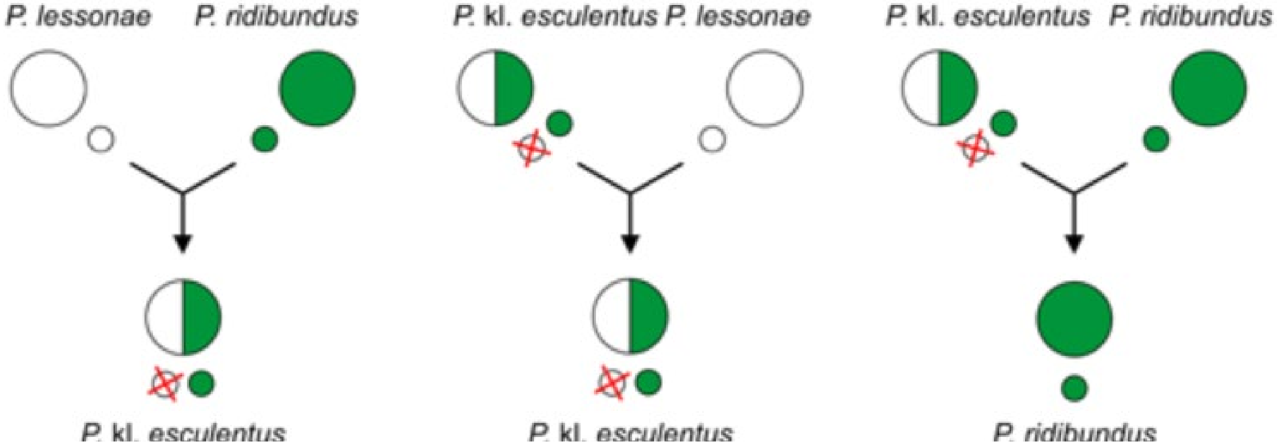
Does a total measure of reproductive isolation help us understand speciation? I don’t think so. Easier to think of reproductive isolation as a kind of balance between gene flow and divergent selection

Hybridogenesis in European frogs

Pelophylax lessonae
The pool frog

Pelophylax “klepton” *esculentus*
The edible frog
Hybrid pool x marsh frog

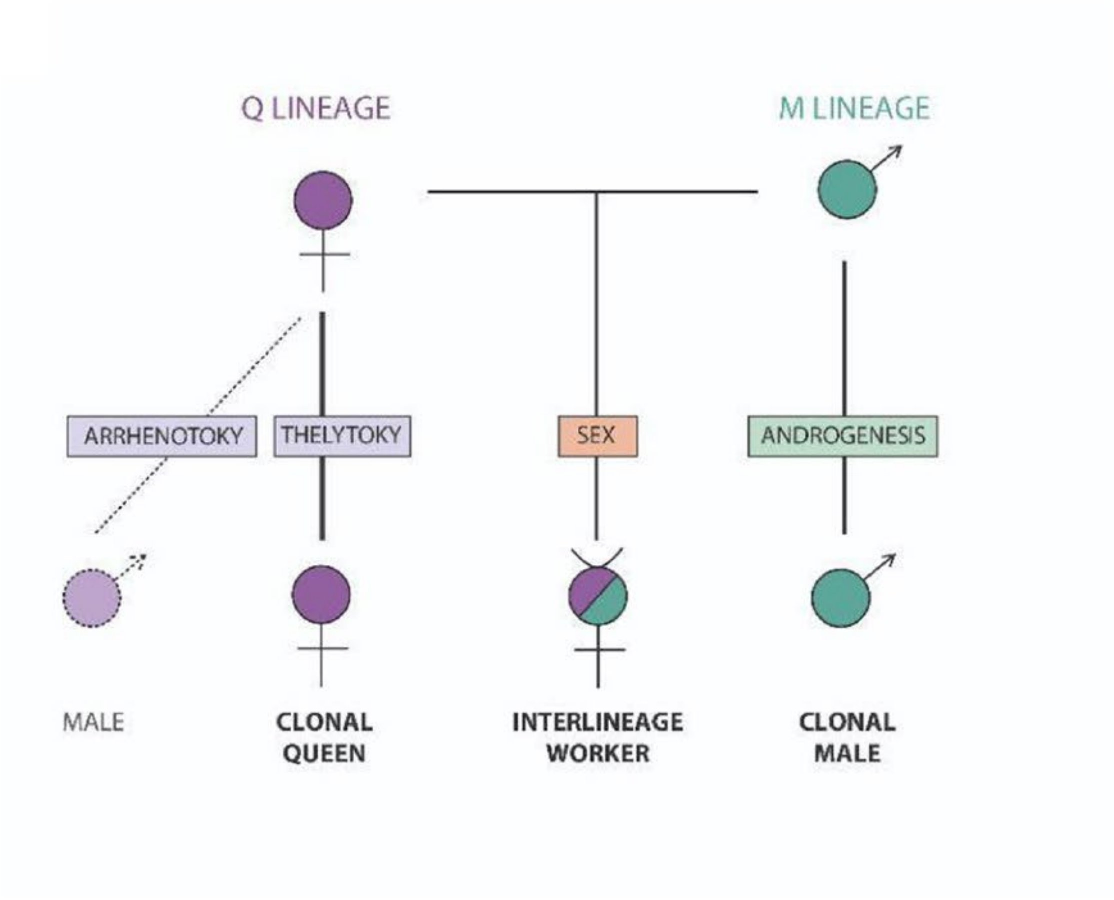
Pelophylax ridibunda
The marsh frog

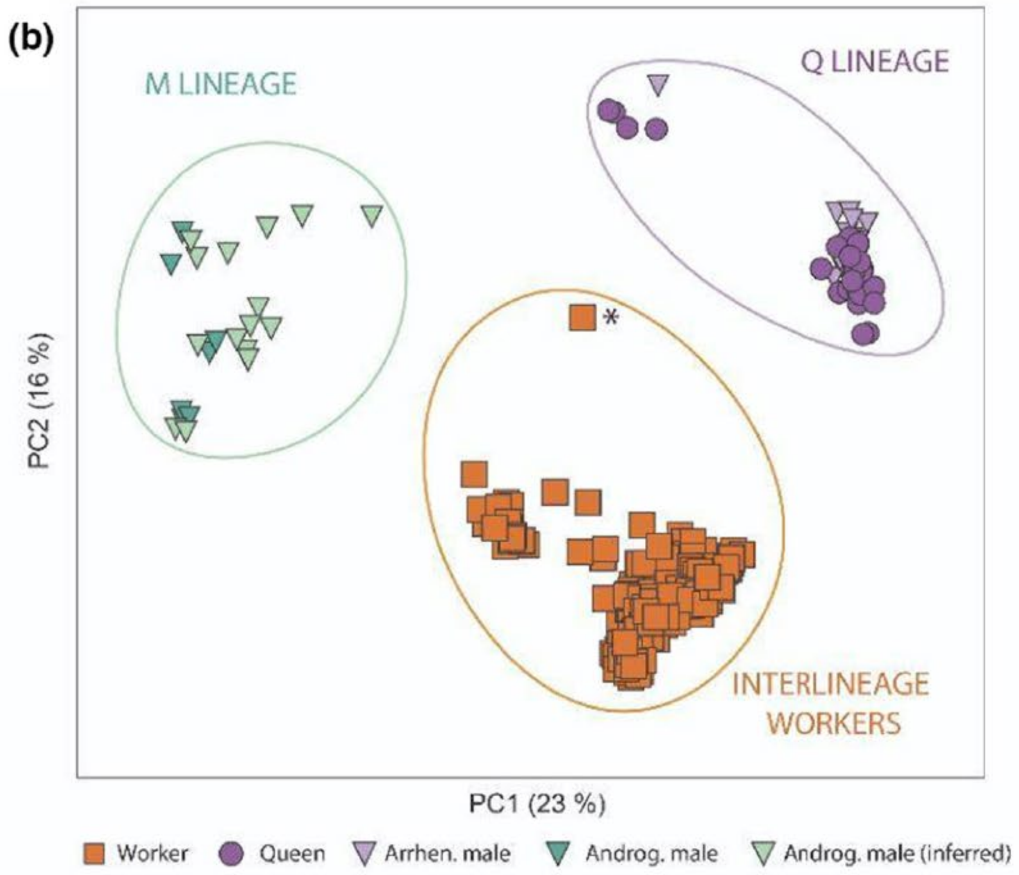


Hybridogenesis also occurs in some fishes and stick insects

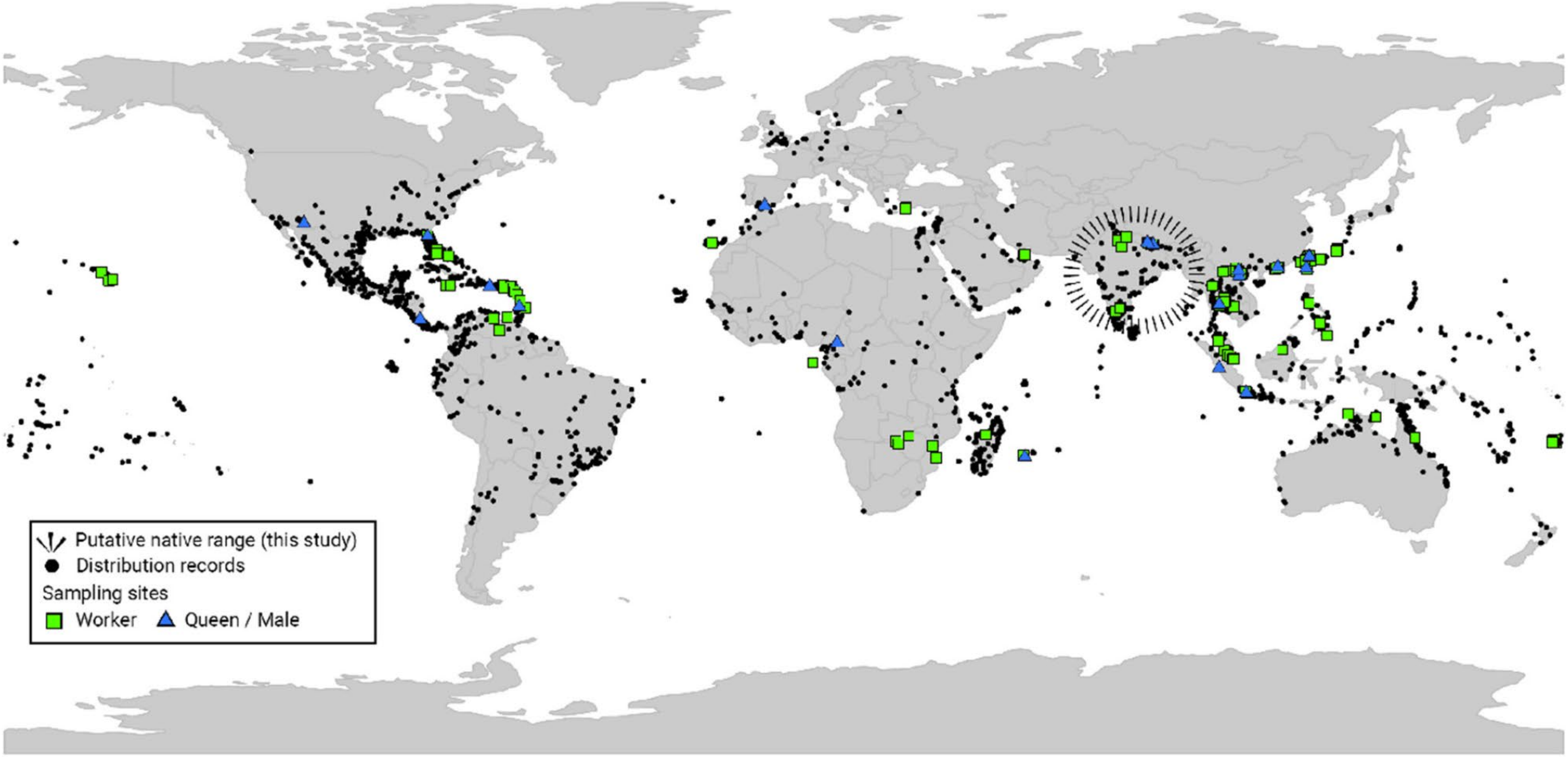
Male and female clonality; worker heterozygosity. *Paratrechina longicornis* ants

- The longhorn crazy ant! See Tseng et al. 2022, and Pearcy 2011.





Paratrechina longicornis distribution



Lastly: Philosophical open questions

Is reproductive isolation a good thing? Most people seem to think so.
Are species "good" because they resist gene flow?

On the one hand, divergence and specialization in ecology seems to require a reduction of gene flow.

On the other hand, wouldn't it be "better" if a species could become a generalist in terms of ecology, and resist divergent specialization and speciation?

Darwinian view: "speciation" is a by-product of natural selection, and not its goal.

Small effective population sizes = less fit; maybe better stay large, or allow some gene flow? (from a species point of view). So species may not be "beneficial."