OEB 140 Speciation

Experimental studies of speciation

Today:

Speciation is usually a long-term process: can it be replicated in the laboratory?

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Experiments…

1) Basic allopatry model of speciation
   Pre- and post-zygotic isolation evolve in allopatry via selection
   Pre- and post-zygotic isolation evolve in allopatry via drift (bottlenecks)
2) Reinforcement
   – allopatry -> low fitness offspring -> reinforcement in sympatry
   drift or selection may lead to divergence
   some reproductive isolation as a by-product
   after contact, selection against hybridization, reinforcement
3) Divergence with gene flow
   – divergent selection in presence of gene flow
   extreme versions: sympatry, or parapatry
   reproductive isolation as a by-product or directly
   caused via disruptive ecological selection

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Advantages and disadvantages of experimental speciation

Experimental Speciation	Comparative methods with natural populations
Rare (but important) serendipitous events are likely to be missed unless the experiment is large	**Better represents the importance of a given process, rather than just its occurrence**
Starting population characteristics and genome are defined or quantified a priori by the researcher	In most cases, it is challenging to reconstruct ancestral populations and their genomes
Typically reliant upon standing variation alone	**Greater potential for de novo mutation or introgression from other populations to play a role**
Environment is controlled and can be kept constant or manipulated in a controlled manner, throughout	Often difficult to determine ancestral environment required to delineate the role of geography in restricting gene flow
Many initial effects may be due to laboratory adaptation. If laboratory adaptation has occurred pre-EE, genetic diversity will be lower	**Populations are typically close to equilibrium in the wild**

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Advantages and disadvantages of experimental speciation (2)

Experimental Speciation	Comparative methods with natural populations
Evolutionary responses are replicated over a series of lines to robustly link conditions to responses	No true replication. Lack of parallelism may create uncertainty that a phenotypic change is a direct response to a given variable
Evolution of traits is limited to what can be performed in culture conditions. Low niche dimensionality means only simple contrasts can be made	**A much wider range of traits can be selected upon or arise**
Gene flow can be more accurately and reliably determined from highly controlled migration levels, and measures of local adaptation and RI	Difficult to determine level of ongoing gene flow *Especially in the past!!!*
Limited to a subset of organisms suitable for [Experimental speciation]	**Can study any diverging populations**
Easy to separate intrinsic and extrinsic forms of RI	Difficult to disentangle intrinsic from extrinsic RI

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Advantages and disadvantages of experimental speciation (3)

Experimental Speciation	Comparative methods with natural populations
Laboratory settings may exclude many of the ecological aspects that separate species	**Can assess the full range of isolating mechanisms found in the wild**
Phenotypic and genomic data can be collected with high temporal resolution providing estimates of phenotypic change and evolutionary hindsight of underlying genomic changes	Even if ancestral genomes can be reconstructed, phenotype data is typically only a single snapshot, so cannot be matched to genomic data
Experiments can only cover short timescales and subsets of the speciation process *Maybe the biggest problem for the experimental approach*	**Long timescales of divergence can be studied (although histories must be inferred).** *Speciation usually inferred to be gradual and to take a long time*

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Experiments on speciation

- Pioneered by Thedosius Dobzhansky using *Drosophila*
- Dobzhansky had worked on beetle taxonomy in Russia, and was interested in speciation. Immigrated to USA in 1927
- Dobzhansky worked in the Thomas Hunt Morgan *Drosophila* laboratory with Sturtevant and Muller, around the time (1933) Morgan won a Nobel Prize for showing that genes resided on chromosomes (in 1911)
- Dobzhansky “took experimental genetics to the field” and did much fieldwork, as well as lab crosses
- Discovered “race A” and “race B” of *Drosophila pseudoobscura*, by means of crosses in the lab
- Concluded “race B” (*D. persimilis*) was a separate species and invented the concept of reproductive isolation
- But never attempted experimental speciation in the lab

Wikipedia

Dobzhansky (web)

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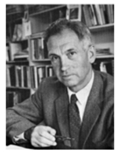
Experiments on speciation

- After New Guinea 1931 Ernst Mayr left Germany for curator position at the American Museum of Natural History, NYC
- Became acquainted with Dobzhansky, then at Columbia, also in New York City
- Mayr had believed in "soft inheritance" (direct influence of the environment on genetics), but Dobzhansky convinced him of the veracity of "hard" Mendelian genetics
- Confined to his home and workplace as an "enemy alien" in WWII, but he performed some *Drosophila* (post-)speciation experiments himself, for example:

Dobzhansky (web)



Mayr ca. 1966 (Hölldobler 2004)

Mayr, E., & Dobzhansky, T. 1945. Experiments on sexual isolation in Drosophila. IV. Modification of the degree of isolation between *Drosophila pseudoobscura* and *Drosophila persimilis* and of sexual preferences in *Drosophila prosaltans*. PNAS 31:75-82

Mayr, E. 1946. Experiments on sexual isolation in *Drosophila*. VII. The nature of the isolating mechanisms between *Drosophila pseudoobscura* and *Drosophila persimilis*. PNAS 32:128-137

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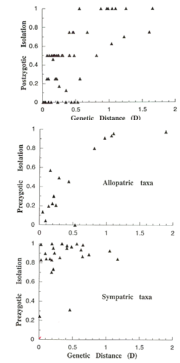
Other lab studies of wild species and isolation

- Coyne & Orr surveyed hybrid inviability/sterility between pairs of related *Drosophila* species.
- Measures were based on wild-collected species.
- Tests on mating behavior and hybrid sterility/inviability in the laboratory.



- Now similar studies done for Lepidoptera, birds, some plants, amphibia
- But this was not laboratory evolution of reproductive isolation

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1) Basic allopatry model of speciation (drift)

a) No selection (drift) e.g. experiments with *Drosophila,* "inbred" = allopatry
Very little evidence of postzygotic isolation among inbred lines
Some assortative mating, but variable, and sometimes negative!

2. Male Choice

	Male	n	Homo-gametic	Hetero-gametic	x^2	x^2 (comb)	Tend.	P.
Inbred	NC-13[1] NC-13	25 25	13 13	7 5	3 3.62	5.24	+	sig.
Inbred irradiated	N20-9[1] N20-9	25 25	12 7	7 8	2.12 .09	.660	+	−
Inbred	NC-11[1] NC-11	25 25	10 14	8 6	.43 5.33	4.406	+	sig.
Inbred irradiated	N20-1[1] N20-1	25 25	11 9	9 9	.33 0	.166	+	−
Inbred	NC-4[1] NC-4	38 37	24 22	11 4	9.133 19.21	27.44	+	sig.
Inbred irradiated	N20-2[1] N20-2	38 37	5 4	8 13	.83 6.18	5.783	−	sig.

(1) Marked female of same type as the male.
(2) Marked males of same type as the female.
−: tendency to heterogametic matings.
+: tendency to homogametic matings.

Koref Santibañez & Waddington 1958

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3. Female Choice

	Female	n	Homo-gametic	Hetero-gametic	x^2	x^2 (comb)	Tend.	P.
Inbred	NC-13[2] NC-13	25 25	5 4	1 3	2.6 .14	2.06	+	−
Inbred irradiated	N20-9[2] N20-9	25 25	11 3	3 7	4 1.6	.27	+	−
Inbred	NC-11[2] NC-11	25 25	5 11	14 8	4.26 .474	.948	−	−
Inbred irradiated	N20-1[2] N20-1	25 25	6 5	2 7	2 .33	.013	+	−
Inbred	NC-4[2] NC-4	25 25	8 12	6 0	.285 12	7.99	+	sig.
Inbred irradiated	N20-2[2] N20-2	25 25	3 0	6 5	1 5	5.28	−	sig.

(1) Marked female of same type as the male.
(2) Marked males of same type as the female.
−: tendency to heterogametic matings.
+: tendency to homogametic matings.

Results: weak, some + and some -

Females not very variable. Main thing is inbreeding makes flies less vigorous. Irradiation makes them even slower to mate

Koref Santibañez & Waddington 1958

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1) Basic allopatry model of speciation (selection)

b) Divergent selection and prezygotic isolation, 16 generations of selection
e.g. house flies (*Musca*) divergently selected for geotaxis, s = 95% in batches of 2000 flies (i.e. 50 flies + or -)
with 0% gene flow, allopatry — strain A + geotaxis
B – geotaxis
with 50% gene flow, sympatry — C + / – geotaxis, randomly mixed

Tests for Homogamic and Heterogamic Matings as a Measure of Reproductive Isolation

	No. of Flies Mated: Male	Female	No. of Matings: Homo	Hetero	Isolation Index (*I*)	χ^2	*P*
Male choice	Test 1:						
"Allopatry"	1 A	1 A 1 B	10	0	1.0	10.6	< .005
	1 B	1 B 1 A	8	2	0.6		
"Sympatry"	1 C(+)	1 C(+) 1 C(−)	10	0	1.0	13.0	< .005
	1 C(−)	1 C(−) 1 C(+)	9	1	0.8		
Male & female choice	Test 2:						
	25 A 25 B	25 A 25 B	25	7	0.56	9.0	< .005
	25 C(+) 25 C(−)	25 C(+) 25 C(−)	29	9	0.52	9.5	< .005

Some sort of pleiotropy between geotaxis and mating behavior. Sympatry not required, nor faster!

Negative results in other experiments

Hurd & Eisenberg 1975

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1) Basic allopatry model of speciation

c) Divergent selection and postzygotic isolation – environment dependent and environment independent
e.g. Drosophila willistoni selected for 49 generations

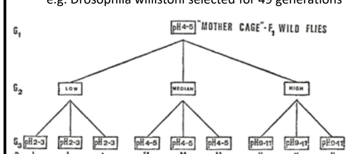

FIG. 1.—Origin of the populations studied. Eighteen cages were used. Procedures for *D. willistoni* from Brasilia were similar to those used for flies from Eldorado. *Abbreviations:* G = generations; B = *D. willistoni* from Brasilia; E = *D. willistoni* from Eldorado; L = populations selected at low *p*H food medium; M = populations selected at intermediate *p*H food medium; H = populations selected at high *p*H food medium.

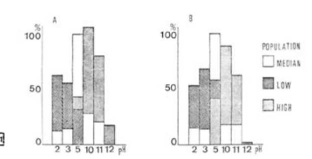

FIG. 2.—Averaged viability of *D. willistoni* populations from Brasilia (A) and Eldorado (B) selected during 49 generations in foods of different *p*H's and tested in different *p*H medium. The average viability in the *p*H 5 bottles was taken as control (100 per cent viability).

Some evidence for significant homogamic preferences. Also some evidence of F1 and F2 hybrids being "reproductively inferior"

de Oliveira & Cordeiro 1980a,b

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2) Reinforcement models

Observational basis of evidence is strong (e.g. Mohamed Noor and *Drosophila pseudoobscura* x *persimilis*

a) "Destroy the hybrids" experiments – but a criticism:
speciation has essentially already been achieved!

Many studies do indeed show increased assortative mating under these conditions. These studies at least show that mating behaviour can be altered.

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2) Reinforcement models

b) Studies allowing gene flow:

Butlin would argue this is a true test of reinforcement ("destroy the hybrids" is not)

Mostly did *not* produce any evidence for assortative mating. One study by Thoday & Gibson (1962) with *Drosophila melanogaster* did find very strong reproductive isolation, but it could not be repeated by most other researchers, including Thoday & Gibson in 1970. Possibly the 1962 result was due to use of heterogeneous stocks from wild strains.

It's the Felsenstein difficulty: Hard to get the linkage disequilibria between genes for mating behavior and genes for adaptation when the mating behaviour is the only adaptation.

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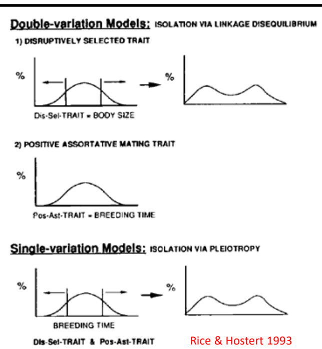
3) "Divergence with gene flow" speciation

Two models of speciation with gene flow

"Felsenstein zone"– LD between assortative mating and disruptively selected trait

Or pleiotropic "magic trait" speciation where the assortative mating trait is also the disruptively selected trait. The need for LD is bypassed by pleiotropy.

(Not a very informative image from Rice & Hostert?)



Rice & Hostert 1993

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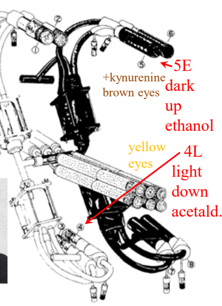
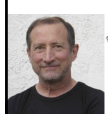
3) "Divergence with gene flow" speciation,





Apparatus selects for phototaxis, geotaxis, chemotaxis and development time. "Multifarious."

Light – dark
Up – down
Ethanol – acetaldehyde (dark vs. light little tube)
Early emergence – late emergence (over 5 days)

Disruptive selection: collect 5 Early and 4 Late
Double selection: also select against females that switched environment (s ~ 50%, males OK)
Flies are *vermilion/raspberry* yellow eye mutants
5E medium + kynurenine -> brown eyes
Females only mate once they have found food.

Rice & Salt 1988, 1990

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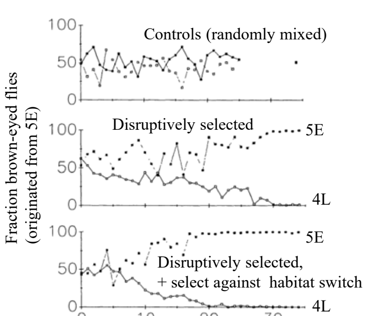

Rice & Salt 1990

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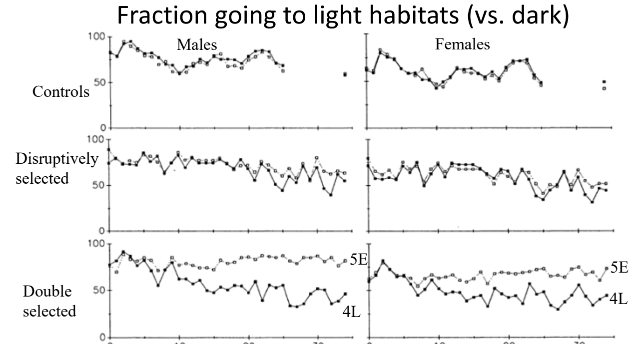
Fraction going to light habitats (vs. dark)



Rice & Salt 1990

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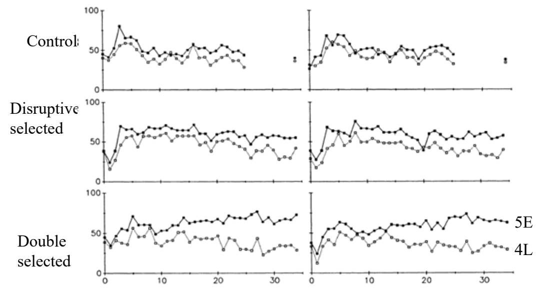
Fraction going to upper habitats (vs. down)



3/27/2025 Rice & Salt 1990 19

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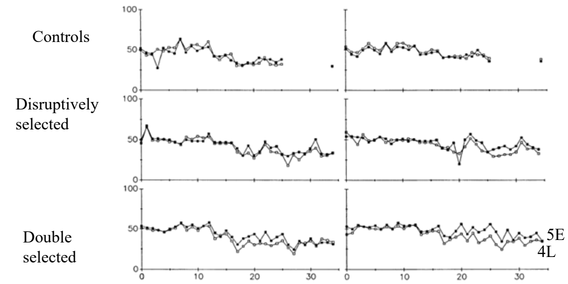
Fraction going to acetaldehyde (vs. ethanol)



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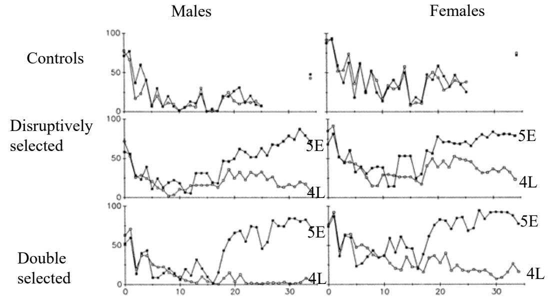
Fraction going to “early” habitats (vs. “late”)



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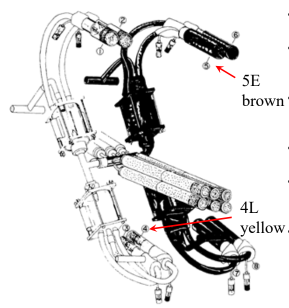
Rice & Salt Drosophila experiment



- Strong multifarous selection to discrete environments led to almost complete reproductive isolation in sympatry
- “Disruptive selection” on many traits led to this mating isolation, because released while immature, and mated only on food.
- It was selection, not allopatry that caused speciation (controls had just as much spatial separation, but no reproductive isolation)
- 50% selection against incorrect habitat choice intensified the selection.
- The effect of each habitat contrast separately was much less than the total effect. “Roughly multiplicative” total effect
- Authors argue this kind of “magic trait” selection is realistic & gets around the Felsenstein problem of need for linkage disequilibria between behavior and survival.

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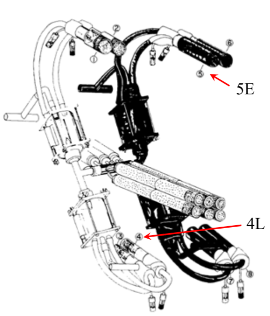
Rice & Salt Drosophila experiment



“... Mating patterns under ‘forced consolidation’ did not change over the course of the experiment....”

Therefore “any prezygotic reproductive isolation evolving in the ... the experiment is solely a consequence of habitat specialization.”

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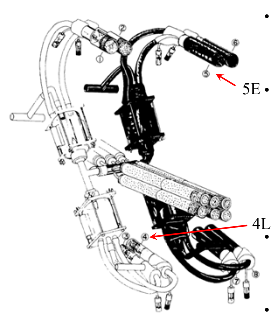
Coyne & Orr 2004 critique



- Selection was too strong; unlikely in natural populations (Rice & Salt argue it’s realistic, e.g. host switching in *Rhagoletis*).
- Coyne & Orr argue it’s effectively “allopatry in a small area”. “Eliminating all individuals” that chose wrong (in the double selection lines) was preventing gene flow. So the double selection is effectively “an allopatric model.” (Coyne & Orr seem to miss the fact that only females were selected out if they chose wrong; so actually there is gene flow here via the males.).
- “Surveying all relevant experiments, ... we conclude that strong disruptive selection is necessary but not sufficient for the evolution of reproductive isolation in sympatry”
- They don’t rate the pleiotropic “magic trait” as important.

3/27/2025 Coyne & Orr 2004: 141 24

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Most of the ideas I got from:

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LABORATORY EXPERIMENTS ON SPECIATION: WHAT HAVE WE LEARNED IN 40 YEARS?

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Abstract.—We integrate experimental studies attempting to duplicate all or part of the speciation process under controlled laboratory conditions and ask what general conclusions can be made concerning the major models of speciation. Strong support is found for the evolution of reproductive isolation via pleiotropy and/or genetic hitchhiking with or without allopatry. Little or no support is found for the bottleneck and reinforcement models of speciation. We conclude that the role of geographical separation in generating allopatry (i.e., zero gene flow induced by spatial isolation) has been overemphasized in the past, whereas its role in generating *diminished* gene flow in combination with strong, discontinuous, and multifarious divergent selection, has been largely unappreciated.

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Lab speciation experiments

Since 1993 there have been a number of new experiments, but I don't think there have been major shifts in understanding as a result.

The major results are:

- Plenty of evidence for divergence in allopatry (i.e. $m = 0$), especially with divergent selection. Some effect on assortative mating ?indirect
- Plenty of evidence for genetic variation available for assortative mating – kill all the hybrids experiments work (effectively in "allopatry")
- Bottleneck (founder effect) experiments don't usually work
- Reinforcement and disruptive selection together experiments don't usually work if there is gene flow
- Strong multifarious disruptive selection "divergence with gene flow" can lead to speciation when there is a pleiotropic effect on mating behaviour, as in Rice and Salt's experiments. Selection against incorrect habitat choice (and therefore mating choice) can strengthen isolation.

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Some references:

Rice, W.R., & Salt, G.W. 1990. The evolution of reproductive isolation as a correlated character under sympatric conditions: experimental evidence. Evolution 44:1140-1152. https://doi.org/10.1111/j.1558-5646.1990.tb05221.x

Rice, W.R., & Hostert, E.E. 1993. Laboratory experiments on speciation: what have we learned in forty years? Evolution 47:1637-1653

Fry, J.D. 2009. Laboratory experiments on speciation, Pages 631-656 *in T. Garland, and M.R. Rose, eds. Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments. University of California Press, Berkeley, CA.* *https://doi.org/10.1525/california/9780520247666.003.0020*

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