

## Supplementary Materials

Table S1. Examples of genes causing intrinsic reproductive isolation (RI), and the extent to which they fit the three criteria for a ‘speciation gene’. HI = intrinsic hybrid inviability, HS = intrinsic hybrid sterility.

Gene	Details of gene and function within species	Study system	Form of RI	Criterion 1: Gene affects RI <sup>1</sup>	Criterion 2: Time of divergence <sup>2</sup>	Criterion 3: Effect size <sup>3</sup>	references
<b>1. <i>OdsH</i></b>	Homeobox gene whose normal function within species involves enhancement of sperm production	<i>Drosophila simulans</i> and <i>D. mauritiana</i>	<b>HS</b>	<b>1: Gene affects RI</b> (causes roughly 50% sterility; other nearby genes required to confer full hybrid sterility)	<b>Unknown</b> (but likely <b>not recent</b> ; <i>OdsH</i> reflects species boundaries more strongly than random loci)	<b>Unknown</b>	[S1-S4]
<b>2. <i>Xmrk2</i></b>	Encodes a receptor tyrosine kinase that is overexpressed in some hybrids	<i>Xiphophorus</i> fish species (e.g. <i>X. maculatus</i> and <i>X. helleri</i> )	<b>HI</b>	<b>1: Gene affects RI</b> (sometimes lethal tumorigenesis in hybrids or embryonic lethality)	<b>Unknown</b> (species easily hybridized in lab)	<b>Unknown</b> (effects on RI vary strongly among crosses)	[S5-S6]
<b>3. <i>Hmr</i></b>	Encodes a protein with	<i>Drosophila</i>	<b>HI,</b>	<b>1: Gene affects</b>	<b>Unknown</b>	<b>Unknown</b>	[S7-S8]

	DNA-binding domains similar to some transcription factors, functional divergence between species shown	<i>simulans</i> and <i>D. melanogaster</i>	<b>HS</b>	<b>RI</b> (affects viability and sterility and fertility, both sexes affected in some way)	(but likely <b>not recent</b> ; changes via positive selection occurred in the fairly distant past)		
<b>4. <i>Lhr</i></b>	Functionally diverged in <i>D. simulans</i> and interacts with <i>Hmr</i> to cause F <sub>1</sub> HI, localizes to heterochromatic genomic regions	<i>Drosophila simulans</i> and <i>D. melanogaster</i>	<b>HI</b>	<b>1: Gene affects RI</b> (contributes to lethality in F <sub>1</sub> hybrid males, other genes might be involved in causing full lethality)	<b>Unknown</b>	<b>Unknown</b>	[S9]
<b>5. <i>Nup96</i></b>	Encodes a nuclear pore protein	<i>Drosophila simulans</i> and <i>D. melanogaster</i>	<b>HI</b>	<b>1: Gene affects RI</b> (explains complete hybrid lethality)	<b>Unknown</b> (but likely <b>not recent</b> ; changes via positive selection occurred in the fairly distant	<b>Unknown</b>	[S10]

					past)		
<b>6. Prdm9</b>	Encodes a histone H3 lysine 4 trimethyltransferase	<i>Mus musculus</i> house mouse subspecies	<b>HS</b>	<b>1: Gene affects RI</b> (affects hybrid sterility)	<b>Before complete RI</b> (RI between subspecies is known to be incomplete)	<b>Large</b> (hybrid sterility effects are strong and RI via other barriers is incomplete)	[S11]
<b>7. JYAlpha</b>	On the 4th chromosome of <i>D. melanogaster</i> but on the 3rd chromosome of <i>D. simulans</i>	<i>Drosophila simulans</i> and <i>D. melanogaster</i>	<b>HS</b>	<b>1: Gene affects RI</b> (strong effects on hybrid male sterility)	<b>Unknown</b> (but <b>not recent</b> ; a relatively ancient gene transposition)	<b>Unknown</b>	[S12]
<b>8. Overdrive</b>	Causes segregation distortion in F1 hybrids as well as RI	<i>Drosophila pseudoobscura</i> subspecies	<b>HS</b>	<b>1: Gene affects RI</b> (causes hybrid male sterility)	<b>Before complete RI</b>	<b>Large</b> (as hybrid sterility effects are strong and RI due to other barriers is incomplete)	[S13]
<b>9. Aep2</b>	Mitochondrial protein encoded on chromosome 13,	<i>Saccharomyces bayanus</i> and <i>S. cerevisiae</i> yeast	<b>HS</b>	<b>1: Gene affects RI</b> (likely causes	<b>Unknown</b>	<b>Unknown</b>	[S14]

	interacts with a mitochondrial gene to cause HS	species		complete sterility)			
<b>10. NB-LRR</b>	Disease resistance gene homolog	<i>Arabidopsis thaliana</i> populations	<b>HI</b>	<b>1: Gene affects RI</b> (affects hybrid necrosis)	<b>Before complete RI</b>	<b>Large</b> (can cause severe HI and RI due to other barriers is incomplete)	[S15]
<b>11. Nup160</b>	Encodes a nuclear pore complex protein, and the protein encoded by <i>Nup160</i> interacts with that of <i>Nup96</i>	<i>Drosophila simulans</i> and <i>D. melanogaster</i>	<b>HI</b>	<b>1: Gene affects RI</b> (affects hybrid lethality)	<b>Unknown;</b> (no evidence for recent selective sweeps)	<b>Unknown</b>	[S16]
<b>12. PPR genes</b>	Cluster of pentatricopeptide repeat genes, within a QTL for cytonuclear hybrid incompatibility	<i>Mimulus guttatus</i> and <i>M. nasutus</i>	<b>HS</b>	<b>2: Gene associated with RI</b>	<b>Before complete RI;</b> (hybrids frequently observed, molecular data show gene flow)	<b>Unknown</b> (QTL cause strong sterility, but prezygotic isolation is also strong)	[S17-S18]
<b>13. <i>cytc</i></b>	A mitochondrial gene which interacts with nuclear genes to cause	Crosses between allopatric populations of	<b>HI</b>	<b>2: Gene associated with RI</b>	<b>Before complete RI</b> (causes RI in	<b>Unknown</b>	[S19-S20]

	hybrid breakdown	<i>Tigriopus californicus</i> copepods		(but HI variable among crosses and environmental conditions)	intraspecific crosses between populations)		
<b>14. infertility QTL</b>	QTL, genes not known	<i>Lycopersicon esculentum</i> and <i>L. hirsutum</i>	<b>HS</b>	<b>4: Gene unknown, RI known</b>	<b>Unknown</b>	<b>Unknown</b>	[S21]

<sup>1</sup>**Criterion 1.** Evidence that a gene affects RI. The four classes of evidence discussed in the text are evaluated. **1: Gene affects RI.** Includes cases in which positional cloning, gene expression, gene knockout, phenotypic effects of mutations in the gene, or transgenics provide evidence that the gene in question causally affects RI. **2: Gene associated with RI.** A known gene is associated with RI, but the genetic evidence is correlative such that another gene could be causing the association. **3: Gene known, RI only inferred.** Strong evidence is available that a gene affects a phenotypic trait under divergent selection, but effects on RI are indirectly inferred from divergent selection. **4: Gene unknown, RI known.** Reproductive isolation maps to a genomic region, but the specific gene(s) are yet to be identified.

<sup>2</sup>**Criterion 2.** Evidence that divergence in the gene occurred prior to the evolution of complete reproductive isolation. **Before complete RI:** Includes intraspecific comparisons and cases in which recurrent hybridization occurs between species. **Not recent:** Includes old species exhibiting complete reproductive isolation for which phylogenetic, population genetic or other analyses suggest divergence in the candidate speciation gene was not recent.

<sup>3</sup>**Criterion 3.** Effect size of the gene. **Large:** Includes cases in which contributions of the gene to current day reproductive isolation are detectable and considerable, and speciation is not yet complete.

Table S2. Examples of candidate speciation genes affecting forms of reproductive isolation other than intrinsic postmating isolation, with evidence for the three ‘speciation gene’ criteria summarized.

Gene	Details of gene or phenotype affected	Study system	Putative Form of RI	Criterion 1: Gene affecting RI	Criterion 2: Timing of divergence	Criterion 3: Increase in total RI	references
<b>1. <i>OBP57d</i> and <i>OBP57e</i></b>	Odorant binding proteins which affect test perception and host plant preference	<i>D. melanogaster</i> and <i>D. sechellia</i> (the latter has evolved distinct physiological and behavioral adaptations to a unique host plant)	<b>DHP</b>	<b>1: Gene affects RI</b> (affects host plant preference)	<b>Unknown</b>	<b>Unknown</b>	[S22]
<b>2. <i>Eda</i></b>	Lateral plate number (in part, adaptation to predation regimes)	<i>Gasterosteus aculeatus</i> freshwater and marine stickleback fishes	<b>II, EDHF</b>	<b>3: Gene known, RI only inferred</b> (some genetic evidence for divergent selection)	<b>Before complete RI</b> (RI between marine and freshwater forms incomplete)	<b>Unknown</b>	[S23-S25]
<b>3. <i>Pitx1</i></b>	Pelvic spine apparatus (in part, adaptation	<i>Gasterosteus aculeatus</i> freshwater and	<b>II, EDHF</b>	<b>3: Gene known, RI only inferred</b>	<b>Before complete RI</b> (as for <i>Eda</i> )	<b>Unknown</b>	[S26-S27]

	to predation regimes)	marine stickleback fishes		(effect on RI could be small)			
<b>4. <i>Mc1r</i></b>	Pigmentation (adaptation to be camouflaged against visual predation)	<i>Peromyscus poliontos</i> mainland and beach oldfield mouse subspecies	<b>II, EDHF</b>	<b>3: Gene known, RI only inferred</b> (effect on RI could be small and effects on phenotype vary among traits)	<b>Before complete RI</b>	<b>Unknown</b>	[S28]
<b>5. <i>Agouti</i></b>	Pigmentation (adaptation for crypsis via visual predation)	<i>Peromyscus poliontos</i> beach mouse subspecies	<b>II, EDHF</b>	<b>3or 4: Gene associated with traits, linked genes could contribute, RI only inferred</b>	<b>Before complete RI</b>	<b>Unknown</b>	[S29]
<b>6. <i>LWS Opsin</i></b>	Vision (visual adaptation to different light environments)	<i>Pundamilia pundamilia</i> and <i>P. nyererei</i> cichlid fishes	<b>II, EDHF, possibly DHP and SI</b>	<b>3: Gene known, RI only inferred</b> (but mixed experimental evidence for	<b>Before complete RI</b> (relatively common hybridization known)	<b>Unknown</b>	[S30]

				RI)			
<b>7. ROSEA</b> (also <b>ELUTA</b> , <b>SULFUREA</b> )	Flower color (attractiveness and adaptation to different pollinators)	<i>Antirrhinum majus</i> <i>striatum</i> and <i>Antirrhinum m.</i> <i>pseudomajus</i> flower morphs	<b>II</b> , <b>EDHF</b>	<b>3: Gene</b> <b>known, RI</b> <b>only inferred</b>	<b>Before complete</b> <b>RI</b> (hybridization known)	<b>Unknown</b>	[S31]
<b>8. Wingless</b> (QTL)	Mimetic wing color and mate preference	<i>Heliconius cydno</i> and <i>H. pachinus</i> mimetic butterflies	<b>II</b> , <b>EDHF</b> , <b>SI</b>	<b>2: Gene</b> <b>associated with</b> <b>RI</b> (gene is within a QTL for RI)	<b>Before complete</b> <b>RI</b> (hybridization known)	<b>Unknown</b>	[S32]
<b>9. CDPK</b> (QTL)	Salt tolerance (adaptation to different soil salinities)	<i>Helianthus</i> sunflower species	<b>II</b>	<b>2: Gene</b> <b>associated with</b> <b>RI</b> (gene is within a QTL for RI)	<b>Unknown</b>	<b>Unknown</b>	[S33]
<b>10. Tpi</b> (QTL)	Perhaps diapause development timing differences	Pheromone and diapause races of <i>Ostrinia nubilalis</i> corn borers	<b>TI</b>	<b>2: Gene</b> <b>associated with</b> <b>RI</b> (gene is within a QTL for RI)	<b>Before complete</b> <b>RI</b> (hybridization known, see also Fig. 3)	<b>Unknown</b>	[S34]
<b>11. Lysin</b>	Sperm protein involved in	<i>Heliothis rufescens</i> and <i>H. corrugata</i>	<b>GI</b>	<b>2: Gene</b> <b>associated with</b>	<b>Before complete</b> <b>RI</b>	<b>Unknown</b>	[S35- S37]

	fertilization	abalone species		<b>RI</b> (but site-directed mutagenesis suggests causality)	(hybridization in the wild known, but appears rare, indicating strong RI)		
<b>12. <i>Bindin</i></b>	Sperm protein involved in fertilization	<i>Echinometra mathaei</i> populations, but also implicated in many other sea urchin taxa		<b>2: Gene associated with RI</b>	<b>Before complete RI for <i>E. mathaei</i></b> populations (but unknown for other taxa, where hybridization appears very rare)	<b>Unknown</b>	[S36-S40]
<b>13. <i>Mc1r</i></b>	Plumage color differences	Incipient species of <i>Monarcha castaneiventris</i> flycatchers	<b>SI</b>	<b>2: Gene associated with RI</b>	<b>Before complete RI</b>	<b>Unknown</b>	[S41-S42]
<b>14. <i>pgFAR</i></b>	Pheromone production	Z and E pheromone races of <i>Ostrinia nubilalis</i>	<b>SI</b>	<b>1: Gene affects RI</b> (affects pheromones known to cause SI)	<b>Before complete RI</b>	<b>Unknown</b> (other reproductive barriers do exist)	[S43]
<b>15. <i>YUP</i></b>	Flower color	<i>Mimulus lewisii</i> and	<b>II,</b>	<b>4: Gene</b>	<b>Unknown</b>	<b>Unknown</b>	[S44-

<b>(QTL)</b>	(attractiveness and adaptation to different pollinators)	<i>M. cardinalis</i> monkeyflower species	<b>EDHF, PI</b>	<b>unknown, RI known</b>	(RI is strong but incomplete, hybrids are fertile)	(but see Box 1 for range of possibilities)	S46]
<b>16. <i>Pe</i> and <i>Pr</i> (QTL)</b>	Performance on ( <i>Pe</i> ) and preference for ( <i>Pr</i> ) different hosts plant species	<i>Acyrtosiphon pisum</i> host races on Alfalfa versus Clover	<b>II, EDHF, DHP</b>	<b>4: Gene unknown, RI known</b>	<b>Before complete RI</b>	<b>Large</b> (as barriers other than <i>Pe</i> and <i>Pr</i> are weak)	[S47-S48]
<b>17. <i>Coy-2</i> (QTL)</b>	Reinforced mating discrimination	<i>D. pseudoobscura</i> and its sibling species <i>D. persimilis</i>	<b>SI</b>	<b>4: Gene unknown, RI known</b> (but 30 candidate genes reside within the QTL)	<b>Before complete RI</b> (hybridization known)	<b>Unknown</b>	[S49]
<b>18. Song and Preference (QTL)</b>	Male song and female acoustic preference for song	<i>Laupala kohalensis</i> and <i>L. paranigra</i> crickets	<b>SI</b>	<b>4: Gene unknown, RI known</b>	<b>Unknown</b>	<b>Unknown</b>	[S50]
<b>19. X-linked gene (QTL)</b>	Behavioral isolation via	Japan and Pacific ocean forms of	<b>SI (also some)</b>	<b>4: Gene unknown, RI known</b>	<b>Before complete RI</b>	<b>Unknown</b>	[S51]

	male behavior	<i>Gasterosteus aculeatus</i> stickleback fish	<b>effect on HS)</b>	<b>known</b>	(hybridization known)		
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See Online Table 1 for explanations of criteria and categories. II = Immigrant Inviability (ecologically-based selection against migrants between environments), EDHF = ecologically-dependent hybrid fitness, PI = pollinator isolation (= sexual isolation in plants), SI = sexual isolation, DHP = divergent habitat preferences, which contribute to habitat isolation, TI = temporal isolation. GI = gametic isolation.

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