

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

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

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

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

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

¹**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.

²**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.

³**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 |
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| 1. <i>OBP57d</i> and <i>OBP57e</i> | 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) | DHP | 1: Gene affects RI (affects host plant preference) | Unknown | Unknown | [S22] |
| 2. <i>Eda</i> | Lateral plate number (in part, adaptation to predation regimes) | <i>Gasterosteus aculeatus</i> freshwater and marine stickleback fishes | II, EDHF | 3: Gene known, RI only inferred (some genetic evidence for divergent selection) | Before complete RI (RI between marine and freshwater forms incomplete) | Unknown | [S23-S25] |
| 3. <i>Pitx1</i> | Pelvic spine apparatus (in part, adaptation | <i>Gasterosteus aculeatus</i> freshwater and | II, EDHF | 3: Gene known, RI only inferred | Before complete RI (as for <i>Eda</i>) | Unknown | [S26-S27] |

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

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

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

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

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| | male behavior | <i>Gasterosteus aculeatus</i> stickleback fish | effect on HS) | known | (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.

Supplementary References

- S1 Perez, D.E. and Wu, C.I. (1995) Further characterization of the Odysseus locus of hybrid sterility in *Drosophila* - one gene is not enough. *Genetics* 140, 201-206
- S2 Ting, C.T., *et al.* (2000) The phylogeny of closely related species as revealed by the genealogy of a speciation gene, Odysseus. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5313-5316
- S3 Ting, C.T., *et al.* (1998) A rapidly evolving homeobox at the site of a hybrid sterility gene. *Science* 282, 1501-1504
- S4 Sun, S., *et al.* (2004) The normal function of a speciation gene, Odysseus, and its hybrid sterility effect. *Science* 305, 81-83
- S5 Wittbrodt, J., *et al.* (1989) Novel putative receptor kinase encoded by the melanoma-inducing tu locus in *Xiphophorus*. *Nature* 341, 415-421
- S6 Schartl, A., *et al.* (1994) Evolutionary origin and molecular-biology of the melanoma-inducing oncogene of *Xiphophorus*. *Pigment Cell Research* 7, 428-432
- S7 Barbash, D.A., *et al.* (2004) Functional divergence caused by ancient positive selection of a *Drosophila* hybrid incompatibility locus. *Plos Biology* 2, 839-848
- S8 Barbash, D.A., *et al.* (2003) A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 5302-5307
- S9 Brideau, N.J., *et al.* (2006) Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila*. *Science* 314, 1292-1295
- S10 Presgraves, D.C., *et al.* (2003) Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. *Nature* 423, 715-719
- S11 Mihola, O., *et al.* (2009) A Mouse Speciation Gene Encodes a Meiotic Histone H3 Methyltransferase. *Science* 323, 373-375
- S12 Masly, J.P., *et al.* (2006) Gene transposition as a cause of hybrid sterility in *Drosophila*. *Science* 313, 1448-1450
- S13 Phadnis, N. and Orr, H.A. (2009) A Single Gene Causes Both Male Sterility and Segregation Distortion in *Drosophila* Hybrids. *Science* 323, 376-379
- S14 Lee, H.Y., *et al.* (2008) Incompatibility of Nuclear and Mitochondrial Genomes Causes Hybrid Sterility between Two Yeast Species. *Cell* 135, 1065-1073
- S15 Bomblies, K., *et al.* (2007) Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. *Plos Biology* 5, 1962-1972

- S16 Tang, S.W. and Presgraves, D.C. (2009) Evolution of the *Drosophila* Nuclear Pore Complex Results in Multiple Hybrid Incompatibilities. *Science* 323, 779-782
- S17 Sweigart, A.L., *et al.* (2006) A simple genetic incompatibility causes hybrid male sterility in *mimulus*. *Genetics* 172, 2465-2479
- S18 Barr, C.M. and Fishman, L. (2010) The nuclear component of a cytonuclear hybrid incompatibility in *Mimulus* maps to a cluster of pentatricopeptide repeat (PPR) genes. *Genetics* 184, 455-465
- S19 Willett, C.S. and Burton, R.S. (2001) Viability of cytochrome C genotypes depends on cytoplasmic backgrounds in *Tigriopus californicus*. *Evolution* 55, 1592-1599
- S20 Willett, C.S. and Burton, R.S. (2003) Environmental influences on epistatic interactions: Viabilities of cytochrome c genotypes in interpopulation crosses. *Evolution* 57, 2286-2292
- S21 Moyle, L.C. and Graham, E.B. (2005) Genetics of hybrid incompatibility between *Lycopersicon esculentum* and *L-hirsutum*. *Genetics* 169, 355-373
- S22 Matsuo, T., *et al.* (2007) Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in *Drosophila sechellia*. *Plos Biology* 5, 985-996
- S23 Colosimo, P.F., *et al.* (2005) Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science* 307, 1928-1933
- S24 Barrett, R.D.H., *et al.* (2008) Natural selection on a major armor gene in threespine stickleback. *Science* 322, 255-257
- S25 Marchinko, K.B. (2009) Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. *Evolution* 63, 127-138
- S26 Shapiro, M.D., *et al.* (2004) Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* 428, 717-723
- S27 Chan, Y.F., *et al.* (2010) Adaptive Evolution of Pelvic Reduction in Sticklebacks by Recurrent Deletion of a *Pitx1* Enhancer. *Science* 327, 302-305
- S28 Hoekstra, H.E., *et al.* (2006) A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* 313, 101-104
- S29 Steiner, C.C., *et al.* (2007) Adaptive variation in beach mice produced by two interacting pigmentation genes. *Plos Biology* 5, 1880-1889
- S30 Seehausen, O., *et al.* (2008) Speciation through sensory drive in cichlid fish. *Nature* 455, 620-U623

- S31 Whibley, A.C., *et al.* (2006) Evolutionary paths underlying flower color variation in *Antirrhinum*. *Science* 313, 963-966
- S32 Kronforst, M.R., *et al.* (2006) Linkage of butterfly mate preference and wing color preference cue at the genomic location of *wingless*. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6575-6580
- S33 Lexer, C., *et al.* (2004) Candidate gene polymorphisms associated with salt tolerance in wild sunflower hybrids: implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. *New Phytologist* 161, 225-233
- S34 Dopman, E.B., *et al.* (2005) Consequences of reproductive barriers for genealogical discordance in the European corn borer. *Proc. Natl. Acad. Sci. U. S. A.* 102, 14706-14711
- S35 Lyon, J.D. and Vacquier, V.D. (1999) Interspecies chimeric sperm lysins identify regions mediating species-specific recognition of the abalone egg vitelline envelope. *Developmental Biology* 214, 151-159
- S36 Panhuis, T.M., *et al.* (2006) Rapid evolution of reproductive proteins in abalone and *Drosophila*. *Philos. Trans. R. Soc. B-Biol. Sci.* 361, 261-268
- S37 Palumbi, S.R. (2009) Speciation and the evolution of gamete recognition genes: pattern and process. *Heredity* 102, 66-76
- S38 Palumbi, S.R. (1999) All males are not created equal: Fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12632-12637
- S39 McCartney, M.A. and Lessios, H.A. (2004) Adaptive evolution of sperm binding tracks egg incompatibility in neotropical sea urchins of the genus *Echinometra*. *Mol. Biol. Evol.* 21, 732-745
- S40 Lessios, H.A. (2007) Reproductive isolation between species of sea urchins. *Bulletin of Marine Science* 81, 191-208
- S41 Uy, J.A.C., *et al.* (2009) Plumage and song differences mediate species recognition between incipient flycatcher species of the Solomon Islands. *Evolution* 63, 153-164
- S42 Uy, J.A.C., *et al.* (2009) Difference in Plumage Color Used in Species Recognition between Incipient Species Is Linked to a Single Amino Acid Substitution in the Melanocortin-1 Receptor. *Am. Nat.* 174, 244-254
- S43 Lassance, J.M., *et al.* (2010) Allelic variation in a fatty-acyl reductase gene causes divergence in moth sex pheromones. *Nature* 466, 486-U487
- S44 Bradshaw, H.D. and Schemske, D.W. (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* 426, 176-178

- S45 Schemske, D.W. and Bradshaw, H.D. (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc. Natl. Acad. Sci. U. S. A.* 96, 11910-11915
- S46 Ramsey, J., *et al.* (2003) Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* 57, 1520-1534
- S47 Hawthorne, D.J. and Via, S. (2001) Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* 412, 904-907
- S48 Via, S. and West, J. (2008) The genetic mosaic suggests a new role for hitchhiking in ecological speciation. *Mol. Ecol.* 17, 4334-4345
- S49 Ortíz-Barrientos, D. and Noor, M.A.F. (2005) Evidence for a one-allele assortative mating locus. *Science* 310, 1467-1467
- S50 Shaw, K.L. and Lesnick, S.C. (2009) Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. *Proc. Natl. Acad. Sci. U. S. A.* 106, 9737-9742
- S51 Kitano, J., *et al.* (2009) A role for a neo-sex chromosome in stickleback speciation. *Nature* 461, 1079-108