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Supplementary Materials for

Many functionally connected loci foster adaptive diversification along a neotropical hybrid zone

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The PDF file includes:

Supplementary Text
Figs. S1 to S5
References

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/39/eabb8617/DC1)

Supplementary File

Supplementary Text: d_{xy}/pi as a measure of population differentiation.

We use d_{xy}/pi as a second measure of population differentiation between hybridizing populations in addition to F_{st}. To explain this further, in hybrid zones with frequent hybrids between adjacent populations, nucleotide diversity (*pi*) can decrease for at least two reasons: First, selection can reduce *pi* as less fit haplotypes are purified from the population. Second, where a locus does not pass freely through a hybrid zone, effective population size will decrease relative to sites with uninhibited gene flow due to splitting of the two populations. The loss of effective population size due to population segregation will result in a decline in nucleotide diversity. With gene flow at a locus and assuming that most nucleotide polymorphisms are neutral variants, we expect that d_{xy} would track similarly to *pi*--as *pi* declines, there are fewer pairwise differences, but these polymorphisms are shared freely between populations (see Fig. S5A) and d_{xy}/pi should be approximately 1. Put another way, pairwise divergence between populations should equal pairwise differences within each population when loci admix, independent of the local nucleotide diversity. Where a selected locus is also a barrier to gene flow, both selection and population separation will reduce *pi*. In this scenario, however, d_{xy} could be initially reduced as nucleotide diversity declines while simultaneously increased over time by the lack of gene flow between populations. Thus, we would expect that the relative loss of nucleotide diversity would be greater than any potential reduction in d_{xy}, and d_{xy}/pi would be greater than 1. More intuitively, the available nucleotide diversity at a locus (*pi*), should flow freely between populations without a barrier to gene flow and differ between loci that do not admix. This effect can be clearly seen at the *dome/wash* color patterning locus. Placed on a ratio scale for clarity, elevated d_{xy}/pi around *dome/wash* is seen as the increased loss of nucleotide diversity relative to the reduction in population divergence (Fig. S5B).

Supplementary figures

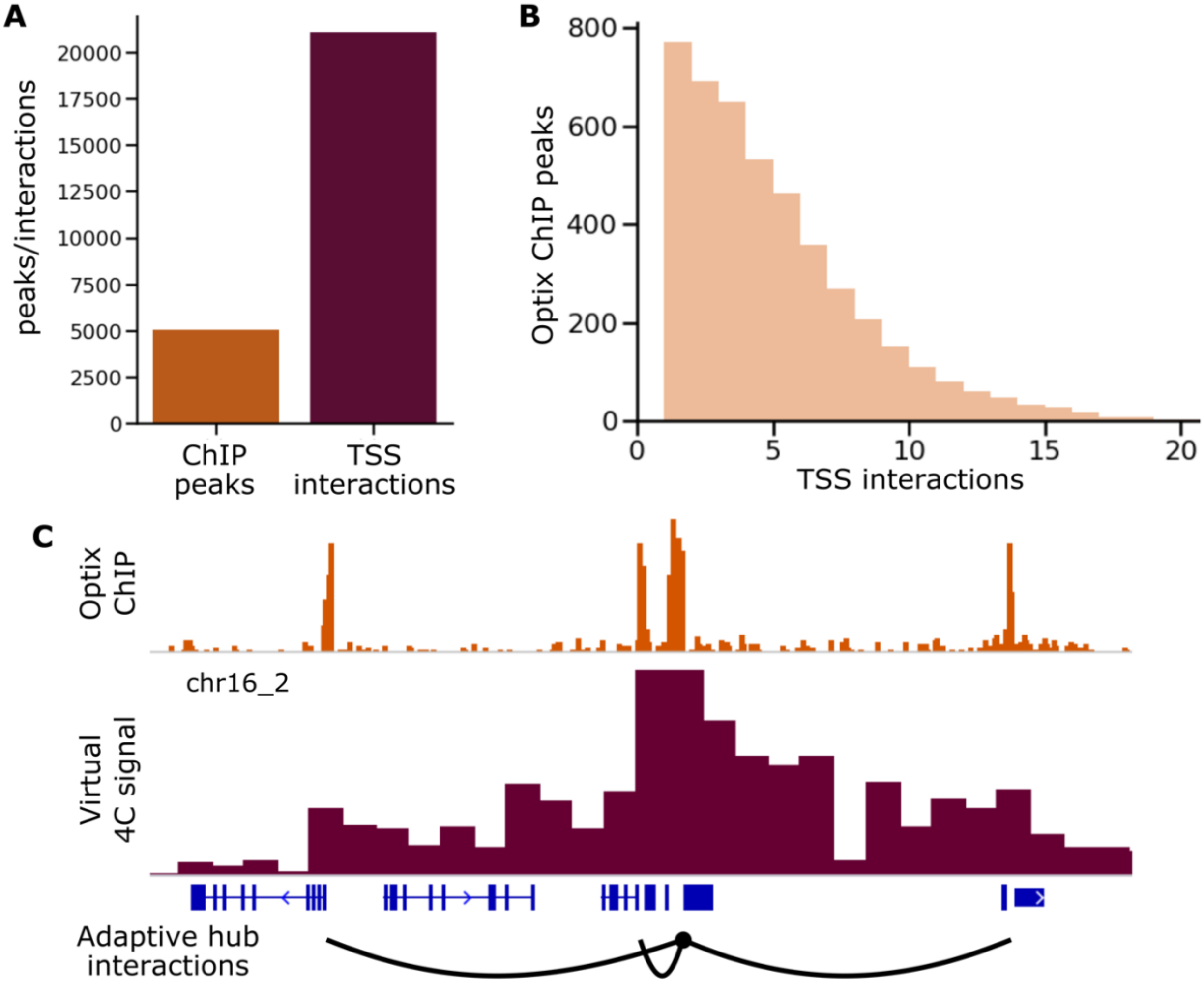

Figure S1. Optix peaks and Hi-C interactions. (**A**) Plot showing the number of ChIP peaks used for whole genome analysis of selection and the count of genome-wide significant Optix peak to TSS interactions. (**B**) Histogram showing the number of significant TSS interactions with Optix ChIP peaks. (**C**) Virtual 4C plot showing Hi-C reads (in 3kb bins) at the adaptive hub shown in Figure 2. Significant TSS interactions drawn below.

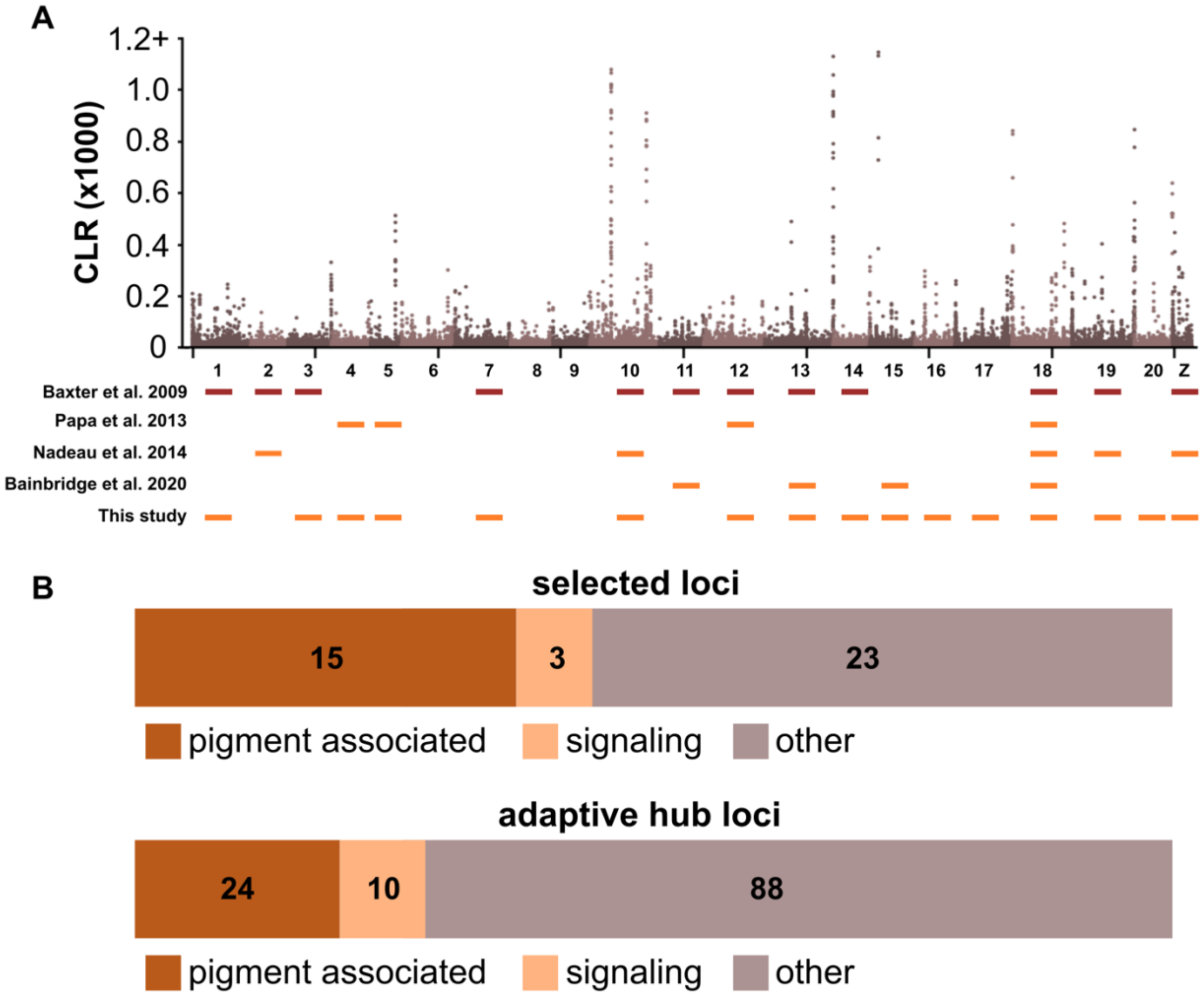

Figure S2. Chromosomes associated with red color pattern evolution and detailed gene function annotations. (**A**) CLR plot for every chromosome with bars below indicating the species (red for *H. melpomene*, orange for *H. erato*) and chromosomes found to significantly associate with red color patterns in three studies of quantitative variation within a population or between hybridizing populations. (**B**) Plots showing the number of genes categorized as pigment associated, signaling, and other for loci with strong signals of selection and adaptive hub genes.

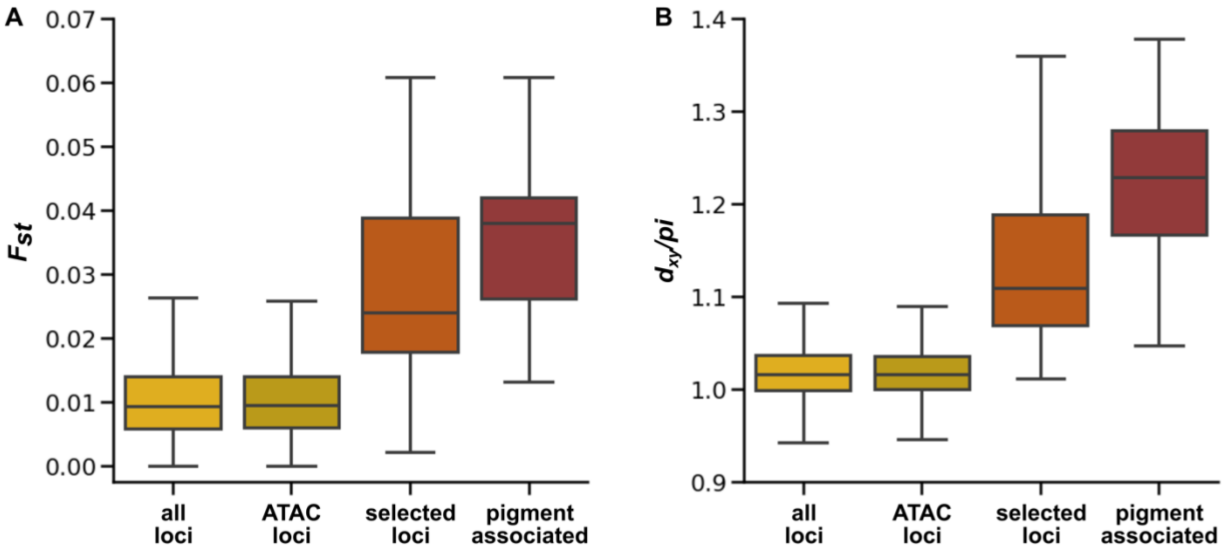

Figure S3. Population differentiation and divergence. Boxplots for (**A**) F_{st} and (**B**) d_{xy}/pi including values for all ATAC peaks in *H. erato* wings at 3 days post-pupation. Boxplots for all windows and values at ATAC peaks are not significantly different.

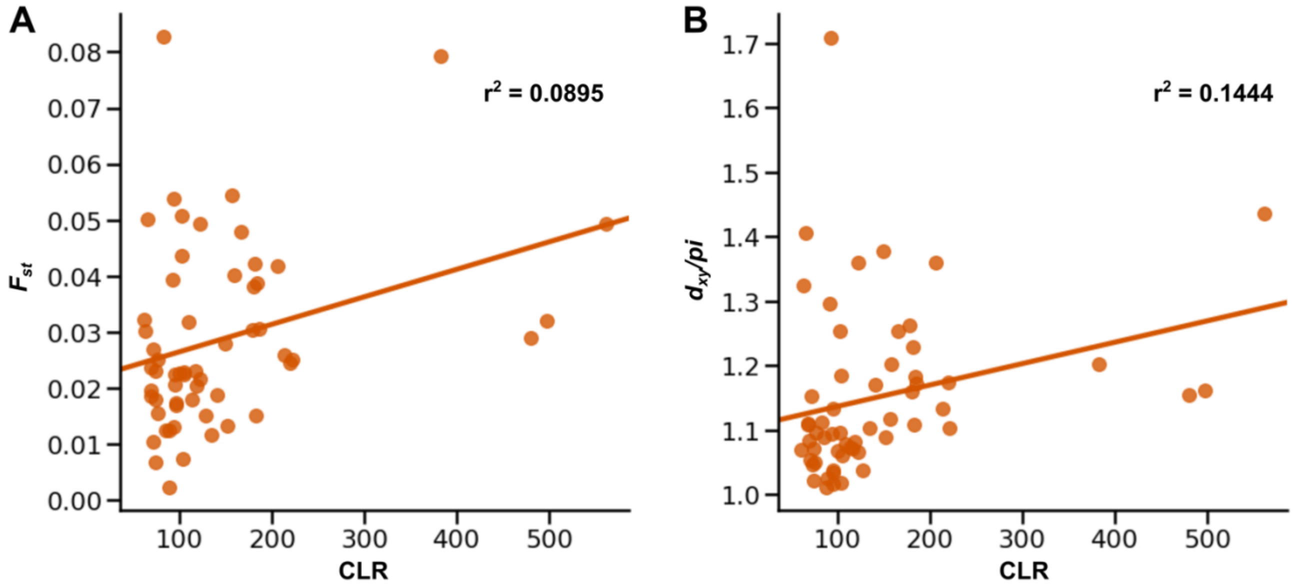

Figure S4. Correlation between CLR and both F_{st} and d_{xy}/pi. Scatterplots showing regression fit line and r-squared (spearman) for CLR against (**A**) F_{st} and (**B**) d_{xy}/pi. A single outlier locus (*dome*/*wash*) was removed from both plots. Neither F_{st} nor d_{xy}/pi is strongly correlated with CLR values, suggesting that observations of differentiation and divergence between populations are not solely the product of selection.

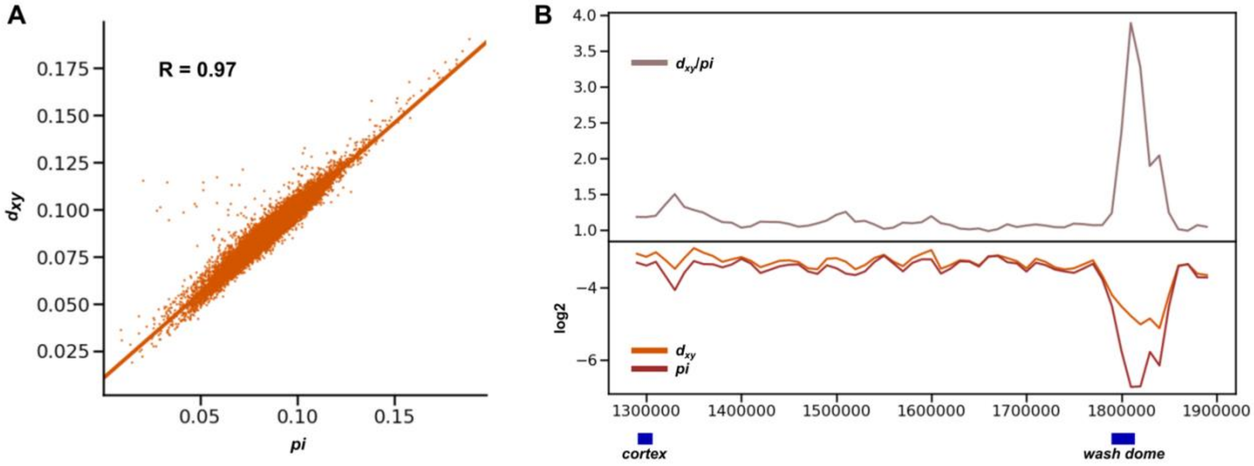

Figure S5. Relationship between d_{xy} and *pi* along a *Heliconius* hybrid zone. (**A**) d_{xy} between Postman and Radiate morphs correlates very strongly with *pi* in the Radiate metapopulation. (**B**) d_{xy}/pi. d_{xy}, and *pi* at the *dome*/*wash* locus.

REFERENCES AND NOTES

1. C. F. Kratochwil, Y. Liang, J. Gerwin, J. M. Woltering, S. Urban, F. Henning, G. Machado-Schiaffino, C. D. Hulsey, A. Meyer, Agouti-related peptide 2 facilitates convergent evolution of stripe patterns across cichlid fish radiations. *Science* **362**, 457–460 (2018).

2. W. A. Cresko, A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. A. Bell, C. B. Kimmel, J. H. Postlethwait, Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 6050–6055 (2004).

3. S. M. Van Belleghem, P. Rastas, A. Papanicolaou, S. H. Martin, C. F. Arias, M. A. Supple, J. J. Hanly, J. Mallet, J. J. Lewis, H. M. Hines, M. Ruiz, C. Salazar, M. Linares, G. R. P. Moreira, C. D. Jiggins, B. A. Counterman, W. O. McMillan, R. Papa, Complex modular architecture around a simple toolkit of wing pattern genes. *Nat. Ecol. Evol.* **1**, 0052 (2017).

4. L. Campagna, M. Repenning, L. F. Silveira, C. S. Fontana, P. L. Tubaro, I. J. Lovette, Repeated divergent selection on pigmentation genes in a rapid finch radiation. *Sci. Adv.* **3**, e1602404 (2017).

5. D. P. L. Toews, S. A. Taylor, R. Vallender, A. Brelsford, B. G. Butcher, P. W. Messer, I. J. Lovette, Plumage genes and little else distinguish the genomes of hybridizing warblers. *Curr. Biol.* **26**, 2313–2318 (2016).

6. J. J. Lee, R. Wedow, A. Okbay, E. Kong, O. Maghzian, M. Zacher, T. A. Nguyen-Viet, P. Bowers, J. Sidorenko, R. K. Linnér, M. A. Fontana, T. Kundu, C. Lee, H. Li, R. Li, R. Royer, P. N. Timshel, R. K. Walters, E. A. Willoughby, L. Yengo; 23andMe Research Team; COGENT (Cognitive Genomics Consortium; Social Science Genetic Association Consortium, M. Alver, Y. Bao, D. W. Clark, F. R. Day, N. A. Furlotte, P. K. Joshi, K. E. Kemper, A. Kleinman, C. Langenberg, R. Mägi, J. W. Trampush, S. S. Verma, Y. Wu, M. Lam, J. H. Zhao, Z. Zheng, J. D. Boardman, H. Campbell, J. Freese, K. M. Harris, C. Hayward, P. Herd, M. Kumari, T. Lencz, J. Luan, A. K. Malhotra, A. Metspalu, L. Milani, K. K. Ong, J. R. B. Perry, D. J. Porteous, M. D. Ritchie, M. C. Smart, B. H. Smith, J. Y. Tung, N. J. Wareham, J. F. Wilson, J. P. Beauchamp, D. C. Conley, T. Esko, S. F. Lehrer, P. K. E. Magnusson, S. Oskarsson, T. H. Pers, M. R. Robinson, K. Thom, C. Watson, C. F. Chabris, M. N. Meyer, D. I. Laibson, J. Yang, M. Johannesson, P. D. Koellinger, P. Turley, P. M.

Visscher, D. J. Benjamin, D. Cesarini, Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nat. Genet.* **50**, 1112–1121 (2018).

7. C. M. Bergey, M. Lopez, G. F. Harrison, E. Patin, J. A. Cohen, L. Quintana-Murci, L. B. Barreiro, G. H. Perry, Polygenic adaptation and convergent evolution on growth and cardiac genetic pathways in African and Asian rainforest hunter-gatherers. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E11256–E11263 (2018).

8. J. P. L. Castro, M. N. Yancoskie, M. Marchini, S. Belohlavy, L. Hiramatsu, M. Kučka, W. H. Beluch, R. Naumann, I. Skuplik, J. Cobb, N. H. Barton, C. Rolian, Y. F. Chan, An integrative genomic analysis of the Longshanks selection experiment for longer limbs in mice. *eLife* **8**, e42014 (2019).

9. M. V. Rockman, The QTN program and the alleles that matter for evolution: All that's gold does not glitter. *Evolution* **66**, 1–17 (2012).

10. E. A. Boyle, Y. I. Li, J. K. Pritchard, An expanded view of complex traits: From polygenic to omnigenic. *Cell* **169**, 1177–1186 (2017).

11. S. M. Van Belleghem, M. Baquero, R. Papa, C. Salazar, W. O. McMillan, B. A. Counterman, C. D. Jiggins, S. H. Martin, Patterns of Z chromosome divergence among *Heliconius* species highlight the importance of historical demography. *Mol. Ecol.* **27**, 3852–3872 (2018).

12. M. A. Supple, R. Papa, H. M. Hines, W. O. McMillan, B. A. Counterman, Divergence with gene flow across a speciation continuum of *Heliconius* butterflies. *BMC Evol. Biol.* **15**, 204 (2015).

13. P. M. Sheppard, J. R. G. Turner, K. S. Brown, W. W. Benson, M. C. Singer, Genetics and the evolution of Muellerian mimicry in *Heliconius* butterflies. *Phil. Trans. R Soc. Lond. B* **308**, 433–610 (1985).

14. J. Morris, N. Navarro, P. Rastas, L. D. Rawlins, J. Sammy, J. Mallet, K. K. Dasmahapatra, The genetic architecture of adaptation: Convergence and pleiotropy in *Heliconius* wing pattern evolution. *Heredity* **123**, 138–152 (2019).

15. L. Zhang, A. Mazo-Vargas, R. D. Reed, Single master regulatory gene coordinates the evolution and development of butterfly color and iridescence. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 10707–10712 (2017).

16. M. A. Supple, H. M. Hines, K. K. Dasmahapatra, J. J. Lewis, D. M. Nielsen, C. Lavoie, D. A. Ray, C. Salazar, W. O. McMillan, B. A. Counterman, Genomic architecture of adaptive color pattern divergence and convergence in *Heliconius* butterflies. *Genome Res.* **23**, 1248–1257 (2013).

17. J. J. Lewis, R. C. Geltman, P. C. Pollak, K. E. Rondem, S. M. Van Belleghem, M. J. Hubisz, P. R. Munn, L. Zhang, C. Benson, A. Mazo-Vargas, C. G. Danko, B. A. Counterman, R. Papa, R. D. Reed, Parallel evolution of ancient, pleiotropic enhancers underlies butterfly wing pattern mimicry. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 24174–24183 (2019).

18. R. Papa, D. D. Kapan, B. A. Counterman, K. Maldonado, D. P. Lindstrom, R. D. Reed, H. F. Nijhout, T. Hrbek, W. O. McMillan, Multi-allelic major effect genes interact with minor effect QTLs to control adaptive color pattern variation in *Heliconius erato*. *PLOS ONE* **8**, e57033 (2013).

19. N. J. Nadeau, M. Ruiz, P. Salazar, B. Counterman, J. A. Medina, H. Ortiz-Zuazaga, A. Morrison, W. O. McMillan, C. D. Jiggins, R. Papa, Population genomics of parallel hybrid zones in the mimetic butterflies. *H. melpomene* and *H. erato*. *Genome Res.* **24**, 1316–1333 (2014).

20. S. W. Baxter, S. E. Johnston, C. D. Jiggins, Butterfly speciation and the distribution of gene effect sizes fixed during adaptation. *Heredity* **102**, 57–65 (2009).

21. H. E. Bainbridge, M. N. Brien, C. Morochz, P. A. Salazar, P. Rastas, N. J. Nadeau, Limited genetic parallels underlie convergent evolution of quantitative pattern variation in mimetic butterflies. *bioRxiv* 10.1101/2020.06.15.151613 (2020).

22. M. DeGiorgio, C. D. Huber, M. J. Hubisz, I. Hellmann, R. Nielsen, SWEEPFINDER2: Increased sensitivity, robustness and flexibility. *Bioinformatics* **32**, 1895–1897 (2016).

23. M. Moest, S. M. Van Belleghem, J. E. James, C. Salazar, S. H. Martin, S. L. Barker, G. R. P. Moreira, C. Mérot, M. Joron, N. J. Nadeau, F. M. Steiner, C. D. Jiggins, Selective sweeps on novel and introgressed variation shape mimicry loci in a butterfly adaptive radiation. *PLOS Biol.* **18**,

e3000597 (2020).

24. D. R. Schrider, A. D. Kern, Soft sweeps are the dominant mode of adaptation in the human genome. *Mol. Biol. Evol.* **34**, 1863–1877 (2017).

25. J. J. Lewis, R. D. Reed, Genome-wide regulatory adaptation shapes population-level genomic landscapes in *Heliconius*. *Mol. Biol. Evol.* **36**, 159–173 (2018).

26. T. Swings, B. Weytjens, T. Schalck, C. Bonte, N. Verstraeten, J. Michiels, K. Marchal, Network-based identification of adaptive pathways in evolved ethanol-tolerant bacterial populations. *Mol. Biol. Evol.* **34**, 2927–2943 (2017).

27. J. Helsen, J. Frickel, R. Jelier, K. J. Verstrepen, Network hubs affect evolvability. *PLOS Biol.* **17**, e3000111 (2019).

28. J. F. Hoyal Cuthill, M. Charleston, Wing patterning genes and coevolution of Müllerian mimicry in *Heliconius* butterflies: Support from phylogeography, cophylogeny, and divergence times. *Evolution* **69**, 3082–3096 (2015).

29. L. Livraghi, J. J. Hanly, L. S. Loh, A. Ren, I. A. Warren, C. Concha, C. Wright, J. M. Walker, J. Foley, H. Arenas-Castro, L. R. Brenes, A. Martin, W. O. McMillan, C. D. Jiggins, The gene *cortex* controls scale colour identity in *Heliconius*. *bioRxiv*:2020.05.26.116533 (2020).

30. N. J. Nadeau, C. Pardo-Diaz, A. Whibley, M. A. Supple, S. V. Saenko, R. W. R. Wallbank, G. C. Wu, L. Maroja, L. Ferguson, J. J. Hanly, H. Hines, C. Salazar, R. M. Merrill, A. J. Dowling, R. H. ffrench-Constant, V. Llaurens, M. Joron, W. O. McMillan, C. D. Jiggins, The gene *cortex* controls mimicry and crypsis in butterflies and moths. *Nature* **534**, 106–110 (2016).

31. J. Mallet, C. Clarke, The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene*. *Proc. R Soc. Lond.* **236**, 163–185 (1989).

32. D. E. Irwin, M. Alcaide, K. E. Delmore, J. H. Irwin, G. L. Owens, Recurrent selection explains parallel evolution of genomic regions of high relative but low absolute differentiation in a ring species. *Mol. Ecol.* **25**, 4488–4507 (2016).

33. T. E. Cruickshank, M. W. Hahn, Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Mol. Ecol.* **23**, 3133–3157 (2014).

34. R. D. Reed, R. Papa, A. Martin, H. M. Hines, B. A. Counterman, C. Pardo-Diaz, C. D. Jiggins, N. L. Chamberlain, M. R. Kronforst, R. Chen, G. Halder, H. F. Nijhout, W. O. McMillan, *optix* drives the repeated convergent evolution of butterfly wing pattern mimicry. *Science* **333**, 1137–1141 (2011).

35. J. Mallet, N. Barton, G. Lamas, J. Santisteban, M. Muedas, H. Eeley, Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in heliconius hybrid zones. *Genetics* **124**, 921–936 (1990).

36. S. Schwartz, M. Truglio, M. J. Scott, H. L. Fitzsimons, Long-term memory in *Drosophila* is influenced by histone deacetylase HDAC4 interacting with sumo-conjugating enzyme Ubc9. *Genetics* **203**, 1249–1264 (2016).

37. J.K. Pritchard, A. Di Rienzo, Adaptation – not by sweeps alone. *Nat. Rev. Genet.* **11**, 665–667 (2010).

38. P. S. Pennings, J. Hermisson, Soft sweeps III: The signature of positive selection from recurrent mutation. *PLOS Genet.* **2**, e186 (2006).

39. P. W. Messer, D. A. Petrov, Population genomics of rapid adaptation by soft selective sweeps. *Trends Ecol. Evol.* **28**, 659–669 (2013).

40. J. Hermisson, P. S. Pennings, Soft sweeps and beyond: Understanding the patterns and probabilities of selection footprints under rapid adaptation. *Methods Ecol. Evol.* **8**, 700–716 (2017).

41. I. Höllinger, P. S. Pennings, J. Hermisson, Polygenic adaptation: From sweeps to subtle frequency shifts. *PLOS Genet.* **15**, e1008035 (2019).

42. F. Dudbridge, Power and predictive accuracy of polygenic risk scores. *PLOS Genet.* **9**, e1003348 (2013).

43. J. J. Lewis, K. R. L. van der Burg, A. Mazo-Vargas, R. D. Reed, ChIP-seq-annotated *Heliconius*

erato genome highlights patterns of cis-regulatory evolution in lepidoptera. *Cell Rep.* **16**, 2855–2863 (2016).

44. B. Langmead, S. L. Salzberg, Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359 (2012).

45. Y. Zhang, T. Liu, C. A. Meyer, J. Eeckhoute, D. S. Johnson, B. E. Bernstein, C. Nusbaum, R. M. Myers, M. Brown, W. Li, X. S. Liu, Model-based analysis of ChIP-seq (MACS). *Genome Biol.* **9**, R137 (2008).

46. S. S. P. Rao, M. H. Huntley, N. C. Durand, E. K. Stamenova, I. D. Bochkov, J. T. Robinson, A. L. Sanborn, I. Machol, A. D. Omer, E. S. Lander, E. L. Aiden, A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665–1680 (2014).

47. N. C. Durand, M. S. Shamim, I. Machol, S. S. P. Rao, M. H. Huntley, E. S. Lander, E. L. Aiden, Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. *Cell Syst.* **3**, 95–98 (2016).

48. J. Ray, P. R. Munn, A. Vihervaara, J. J. Lewis, A. Ozer, C. G. Danko, J. T. Lis, Chromatin conformation remains stable upon extensive transcriptional changes driven by heat shock. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 19431–19439 (2019).

49. G. A. Van der Auwera, M. O. Carneiro, C. Hartl, R. Poplin, G. del Angel, A. Levy-Moonshine, T. Jordan, K. Shakir, D. Roazen, J. Thibault, E. Banks, K. V. Garimella, D. Altshuler, S. Gabriel, M. A. DePristo, From FastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. *Curr. Protoc. Bioinformatics* **43**, 11.10.1–11.10.33 (2013).

50. P. Danecek, A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin; Group 1000 Genomes Project Analysis, The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).

51. C. D. Huber, M. DeGiorgio, I. Hellmann, R. Nielsen, Detecting recent selective sweeps while controlling for mutation rate and background selection. *Mol. Ecol.* **25**, 142–156 (2016).

52. A. R. Quinlan, I. M. Hall, BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).

53. S. H. Martin, M. Möst, W. J. Palmer, C. Salazar, W. O. McMillan, F. M. Jiggins, C. D. Jiggins, Natural selection and genetic diversity in the butterfly *Heliconius melpomene*. *Genetics* **203**, 525–541 (2016).

54. R. Nielsen, S. Williamson, Y. Kim, M. J. Hubisz, A. G. Clark, C. Bustamante, Genomic scans for selective sweeps using SNP data. *Genome Res.* **15**, 1566–1575 (2005).

55. A. Dinwiddie, R. Null, M. Pizzano, L. Chuong, A. Leigh Krup, H. Ee Tan, N. H. Patel, Dynamics of F-actin prefigure the structure of butterfly wing scales. *Dev. Biol.* **392**, 404–418 (2014).

56. R. Rousset, J. A. Mack, K. A. Wharton Jr, J. D. Axelrod, K. M. Cadigan, M. P. Fish, R. Nusse, M. P. Scott, *naked cuticle* targets *dishevelled* to antagonize Wnt signal transduction. *Genes Dev.* **15**, 658–671 (2001).

57. W.-W. Lv, H.-M. Wei, D.-L. Wang, J.-Q. Ni, F.-L. Sun, Depletion of histone deacetylase 3 antagonizes PI3K-mediated overgrowth of *Drosophila* organs through the acetylation of histone H4 at lysine 16. *J. Cell Sci.* **125**, 5369–5378 (2012).

58. C. Drelon, M. F. Rogers, H. M. Belalcazar, J. Secombe, The histone demethylase KDM5 controls developmental timing in *Drosophila* by promoting prothoracic gland endocycles. *Development* **146**, dev182568 (2019).

59. R. T. Collins, J. E. Treisman, Osa-containing Brahma chromatin remodeling complexes are required for the repression of Wingless target genes. *Genes Dev.* **14**, 3140–3152 (2000).

60. A. S. Sowa, E. Martin, I. M. Martins, J. Schmidt, R. Depping, J. J. Weber, F. Rother, E. Hartmann, M. Bader, O. Riess, H. Tricoire, T. Schmidt, Karyopherin α-3 is a key protein in the pathogenesis of spinocerebellar ataxia type 3 controlling the nuclear localization of ataxin-3. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E2624–E2633 (2018).

61. H. R. Byers, M. Yaar, M. S. Eller, N. L. Jalbert, B. A. Gilchrest, Role of cytoplasmic dynein in

melanosome transport in human melanocytes. *J. Invest. Dermatol.* **114**, 990–997 (2000).

62. Z. Lakatos, P. Lőrincz, Z. Szabó, P. Benkő, L. A. Kenéz, T. Csizmadia, G. Juhász, Sec20 is required for autophagic and endocytic degradation independent of Golgi-ER retrograde transport. *Cell* **8**, 768 (2019).

63. E. Y. Seo, S.-P. Jin, K.-C. Sohn, C.-H. Park, D. H. Lee, J. H. Chung, UCHL1 regulates melanogenesis through controlling MITF stability in human melanocytes. *J. Invest. Dermatol.* **137**, 1757–1765 (2017).

64. C. E. Ooi, E. C. Dell'Angelica, J. S. Bonifacino, ADP-ribosylation factor 1 (ARF1) regulates recruitment of the ap-3 adaptor complex to membranes. *J. Cell Biol.* **142**, 391–402 (1998).

65. M. Sasaki, T. Horikoshi, H. Uchiwa, Y. Miyachi, Up-regulation of *Tyrosinase* gene by nitric oxide in human melanocytes. *Pigment Cell Res.* **13**, 248–252 (2000).

66. H. Hayashi, R. Fujii, Possible involvement of nitric oxide in signaling pigment dispersion in teleostean melanophores. *Zoolog. Sci.* **18**, 1207–1215 (2001).

67. L. Zhou, H. Liang, X. Zhou, J. Jia, C. Ye, Q. Hu, S. Xu, Y. Yu, G. Zou, G. Hu, Genetic characteristic and RNA-seq analysis in transparent mutant of carp–goldfish nucleocytoplasmic hybrid. *Genes* **10**, 704 (2019).

68. P. Protiva, J. Gong, B. Sreekumar, R. Torres, X. Zhang, G. S. Belinsky, M. Cornwell, S. E. Crawford, Y. Iwakiri, C. Chung, Pigment epithelium-derived factor (PEDF) inhibits Wnt/β-catenin signaling in the liver. *Cell. Mol. Gastroenterol. Hepatol.* **1**, 535–549.e14 (2015).

69. H. Tang, Z. Kambris, B. Lemaitre, C. Hashimoto, Two proteases defining a melanization cascade in the immune system of *Drosophila*. *J. Biol. Chem.* **281**, 28097–28104 (2006).

70. M. Abrisqueta, C. Herraiz, A. B. Pérez Oliva, B. L. Sanchez-Laorden, C. Olivares, C. Jiménez-Cervantes, J. C. García-Borrón, Differential and competitive regulation of human melanocortin 1 receptor signaling by β-arrestin isoforms. *J. Cell Sci.* **126**, 3724–3737 (2013).

71. M. Tare, A. Sarkar, S. Bedi, M. Kango-Singh, A. Singh, Cullin-4 regulates Wingless and JNK signaling-mediated cell death in the *Drosophila* eye. *Cell Death Dis.* **7**, e2566 (2016).

72. L. F. Sembajwe, K. Katta, M. Grønning, M. Kusche-Gullberg, The exostosin family of glycosyltransferases: mRNA expression profiles and heparan sulphate structure in human breast carcinoma cell lines. *Biosci. Rep.* **38**, BSR20180770 (2018).

73. H. Lee, S.-M. Cheong, W. Han, Y. Koo, S.-B. Jo, G.-S. Cho, J.-S. Yang, S. Kim, J.-K. Han, Head formation requires Dishevelled degradation that is mediated by March2 in concert with Dapper1. *Development* **145**, dev143107 (2018).

74. H. Court, I. M. Ahearn, M. Amoyel, E. A. Bach, M. R. Philips, Regulation of NOTCH signaling by RAB7 and RAB8 requires carboxyl methylation by ICMT. *J. Cell Biol.* **216**, 4165–4182 (2017).

75. I. K. Hariharan, K. Q. Hu, H. Asha, A. Quintanilla, R. M. Ezzell, J. Settleman, Characterization of rho GTPase family homologues in *Drosophila melanogaster*: Overexpressing Rho1 in retinal cells causes a late developmental defect. *EMBO J.* **14**, 292–302 (1995).

76. C. E. Ooi, J. E. Moreira, E. C. Dell'Angelica, G. Poy, D. A. Wassarman, J. S. Bonifacino, Altered expression of a novel adaptin leads to defective pigment granule biogenesis in the *Drosophila* eye color mutant garnet. *EMBO J.* **16**, 4508–4518 (1997).

77. N. Jin, C. Y. Chow, L. Liu, S. N. Zolov, R. Bronson, M. Davisson, J. L. Petersen, Y. Zhang, S. Park, J. E. Duex, D. Goldowitz, M. H. Meisler, L. S. Weisman, VAC14 nucleates a protein complex essential for the acute interconversion of PI3P and PI(3,5)P_2 in yeast and mouse. *EMBO J.* **27**, 3221–3234 (2008).

78. W. Fu, J. Sun, G. Huang, J. C. Liu, A. Kaufman, R. J. H. Ryan, S. Y. Ramanathan, T. Venkatesh, B. Singh, Squamous cell carcinoma-related oncogene (SCCRO) family members regulate cell growth and proliferation through their cooperative and antagonistic effects on cullin neddylation. *J. Biol. Chem.* **291**, 6200–6217 (2016).

79. J. L. Mummery-Widmer, M. Yamazaki, T. Stoeger, M. Novatchkova, S. Bhalerao, D. Chen, G. Dietzl, B. J. Dickson, J. A. Knoblich, Genome-wide analysis of Notch signalling in *Drosophila* by

transgenic RNAi. *Nature* **458**, 987–992 (2009).

80. M. Tsuda, K.-H. Seong, T. Aigaki, POSH, a scaffold protein for JNK signaling, binds to ALG-2 and ALIX in *Drosophila*. *FEBS Lett.* **580**, 3296–3300 (2006).

81. N. G. Lee, Y. K. Hong, S. Y. Yu, S. Y. Han, D. Geum, K. S. Cho, dXNP, a *Drosophila* homolog of XNP/ATRX, induces apoptosis via Jun-N-terminal kinase activation. *FEBS Lett.* **581**, 2625–2632 (2007).

82. K. F. Ketosugbo, H. L. Bushnell, R. I. Johnson, A screen for E3 ubiquitination ligases that genetically interact with the adaptor protein Cindr during *Drosophila* eye patterning. *PLOS ONE* **12**, e0187571 (2017).

83. R. S. Holmes, Comparative studies of vertebrate iduronate 2-sulfatase (*IDS*) genes and proteins: Evolution of A mammalian X-linked gene. *3 Biotech* **7**, 22 (2017).

84. N. Aznar, K. K. Midde, Y. Dunkel, I. Lopez-Sanchez, Y. Pavlova, A. Marivin, J. Barbazán, F. Murray, U. Nitsche, K.-P. Janssen, K. Willert, A. Goel, M. Abal, M. Garcia-Marcos, P. Ghosh, Daple is a novel non-receptor GEF required for trimeric G protein activation in Wnt signaling. *eLife* **4**, e07091 (2015).

85. G. Rosengren Pielberg, A. Golovko, E. Sundström, I. Curik, J. Lennartsson, M. H. Seltenhammer, T. Druml, M. Binns, C. Fitzsimmons, G. Lindgren, K. Sandberg, R. Baumung, M. Vetterlein, S. Strömberg, M. Grabherr, C. Wade, K. Lindblad-Toh, F. Pontén, C.-H. Heldin, J. Sölkner, L. Andersson, A *cis*-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nat. Genet.* **40**, 1004–1009 (2008).

86. H. Kanuka, E. Kuranaga, T. Hiratou, T. Igaki, B. Nelson, H. Okano, M. Miura, Cytosol-endoplasmic reticulum interplay by Sec61α translocon in polyglutamine-mediated neurotoxicity in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 11723–11728 (2003).

87. R. K. Bikkavilli, C. C. Malbon, Arginine methylation of G3BP1 in response to Wnt3a regulates β-catenin mRNA. *J. Cell Sci.* **124**, 2310–2320 (2011).

88. C. A. McLean, A. Lutz, K. J. Rankin, A. Elliott, A. Moussalli, D. Stuart-Fox, Red carotenoids and associated gene expression explain colour variation in frillneck lizards. *Proc. Biol. Sci.* **286**, 20191172 (2019).