

SHAPE OF THE SPECIES BOUNDARY

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Debates about competing concepts of biological species continue almost endlessly, but only rarely have data been used to address the problem^{1,2}. Here we use hybrid specimens from museum collections to analyse the boundaries of species in the wild. *Heliconius* butterflies and their allies have radiated rapidly in tropical America. Their garish colour patterns function as warning and mimicry signals, are highly diversified, and make them popular with collectors. As a result, they are well-represented in collections, and their taxonomy is well-characterised³. Major features of their phylogeny are also understood, thanks to analyses of morphological^{3,4} and DNA sequence data^{5,6}. We surveyed major world museums, private collections, and the literature for specimens of hybrids between species⁷ (here, species are considered to be forms able to maintain multilocus differences in areas of overlap).

Hybridization is generally rare on a per individual basis; hybrid fractions are mostly $< 10^{-3}$ compared with parental individuals^{7,8}. However, many species are involved; 23.5% of the 68 *Heliconiiti* hybridize with at least one other species⁷. The probability of obtaining hybrid specimens should depend on the compatibility of the parent species, but will also be strongly affected by geographic overlap and abundances of parental species, and by our ability to distinguish hybrids, particularly backcrosses, from intraspecific variation. In Fig. 1, the numbers of hybrids are plotted against sequence divergence at a mitochondrial region, COI+COII^{5,6}. While the data is noisy, a increasing numbers of hybrids in the most closely-related species is evident. When estimates of the numbers of intraspecific hybrids in world collections are plotted on the same scale, they do not deviate strongly from the log-linear fit of interspecific hybridizations (Fig. 1), demonstrating continuity between intraspecific compatibility and varying levels of interspecific incompatibility.

Previous work has shown a similar decline in compatibility with genetic distance in *Drosophila*⁹ and bacterial strains (*Bacillus*)¹⁰, but to our knowledge this is the first time that the effect has been demonstrated for natural populations. All hybrids were from three rapidly-radiating and speciose groups³⁻⁶ within the Heliconiiti: the genus *Eueides*, and the *erato/sara* and *melpomene*/silvaniform groups within *Heliconius*. Two pairs of species accounting for over 79% of the hybrids (*H. erato-himera* and *H. melpomene-cydno*) are also among the closest genetically ($\pi=0.030-0.031$).

Our data conform approximately to a log-linear function known as the “exponential failure law”. Previous laboratory work has demonstrated a similar function for transformation between bacterial strains, where the effect is probably due to sensitivity of repair mechanisms to DNA sequence differences¹⁰. The decline in compatibility in butterflies is almost certainly due to different causes, such as assortative mating and hybrid inviability, but our results suggest that a similar log-linear compatibility/divergence function may hold across all of life. Our perception that species are qualitatively different from intraspecific populations is likely to be an artifact due to the difficulty of observing rare hybridizations predicted by this non-linear function, rather than a real discontinuity at the species boundary.

References (Science format)

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Figure legend

Figure 1. The species boundary in heliconiines

The numbers of natural hybrids known between 160 pairs of sympatric species (red points)⁸ are plotted against divergence (π) estimated from 942 bp of mtDNA^{5,6}; known backcrossing is indicated by a halo. A least-squares exponential fit to the untransformed data (including zeroes) is shown. A reduction in hybrids across the species boundary is not in doubt; our interest was in demonstrating the form of this relationship beyond the species boundary, rather than in testing whether the fit is significant (nominally, $P=0.0038$, uncorrected for phylogeny). The numbers of interracial hybrids from 26 polytypic species (not used in the curve fit) in collections worldwide were estimated by multiplying the numbers of intraspecific hybrids documented in a single well-documented collection (W. Neukirchen, University of Florida) by the reciprocal of the fraction of world interspecific hybrids contained in the collection; hybrids known but not documented by the Neukirchen collection were scored as 1.

