

## Neutrality or selection?

In examining a large sample of parent-offspring data from two human major histocompatibility complex loci, *HLA-A* and *HLA-B*, from South Amerindians (F. L. Black and P. W. Hedrick, unpublished results), I have discovered a unique bi-allelic model. In this model there are no changes in allelic frequency for any given initial frequency — a neutral equilibrium — but there is a minimum mean fitness and a maximum excess of heterozygotes over Hardy–Weinberg expectations for equal allelic frequencies.

For all types of mating in the sample (see Table 1) that can produce homozygotes, except where the mother is a homozygote and the father is a heterozygote, there is a significant excess of heterozygotes over expected mendelian proportions, with a large (0.46) and statistically significant selection coefficient (*s*) against homozygotes<sup>1</sup>. These results are suggestive of major histocompatibility complex (MHC) involvement in maternal-fetal interactions where homozygous offspring are selected against when carried by heterozygous mothers.

This selection is described by the bi-allelic model below, where alleles *A*<sub>1</sub> and *A*<sub>2</sub> have frequencies of *p* and *q* and genotypes *A*<sub>1</sub>*A*<sub>1</sub>, *A*<sub>1</sub>*A*<sub>2</sub>, and *A*<sub>2</sub>*A*<sub>2</sub> have frequencies of *P*, *H* and *Q*. Note that Hardy–Weinberg proportions are not assumed and that only homozygous progeny from heterozygous mothers are selected against. The mean fitness is  $\bar{w} = 1 - sH/2$  and the expected change in the frequency of *A*<sub>1</sub> is:

$$\Delta q = \frac{q(q - sH/2) + pq - q\bar{w}}{\bar{w}} = 0.$$

Quite surprisingly, there is no change in allelic frequency for any value of *q* (Fig. 1), just as for the neutrality model, the basis of molecular evolution theory, in which all genotypes have the same fitness.

The equilibrium frequency of heterozygotes is calculated by letting *H*<sub>e</sub> = *H* so that:

$$H_e = \frac{2pq}{(1 - sH_e/2)} = \frac{1 - (1 - 4spq)^{1/2}}{s}$$

The mean fitness at equilibrium is  $\bar{w} = 1 - sH_e/2$ . The equilibrium values of heterozygosity and mean fitness are reached quickly from any starting genotypic frequencies.

The mean fitness is a function of allelic frequencies with a minimum at *p* = *q* and a maximum when *q* = 0 or 1 (Fig. 1), a pattern reminiscent of selection against heterozygotes<sup>2</sup>. The fixation index *F* = 1 - (*H*<sub>e</sub>/2*pq*) is negative (Fig. 1), reminiscent of selection favouring hetero-

**Table 1 Selection model**

Parents		Progeny		
Female × Male	Frequency	<i>A</i> <sub>1</sub> <i>A</i> <sub>1</sub>	<i>A</i> <sub>1</sub> <i>A</i> <sub>2</sub>	<i>A</i> <sub>2</sub> <i>A</i> <sub>2</sub>
<i>A</i> <sub>1</sub> <i>A</i> <sub>1</sub> × <i>A</i> <sub>1</sub> <i>A</i> <sub>1</sub>	<i>P</i> <sup>2</sup>	1	-	-
<i>A</i> <sub>1</sub> <i>A</i> <sub>1</sub> × <i>A</i> <sub>1</sub> <i>A</i> <sub>2</sub>	<i>PH</i>	1	1	-
<i>A</i> <sub>1</sub> <i>A</i> <sub>1</sub> × <i>A</i> <sub>2</sub> <i>A</i> <sub>2</sub>	<i>PQ</i>	-	1	-
<i>A</i> <sub>1</sub> <i>A</i> <sub>2</sub> × <i>A</i> <sub>1</sub> <i>A</i> <sub>1</sub>	<i>PH</i>	1 - <i>s</i>	1	-
<i>A</i> <sub>1</sub> <i>A</i> <sub>2</sub> × <i>A</i> <sub>1</sub> <i>A</i> <sub>2</sub>	<i>H</i> <sup>2</sup>	1 - <i>s</i>	1	1 - <i>s</i>
<i>A</i> <sub>1</sub> <i>A</i> <sub>2</sub> × <i>A</i> <sub>2</sub> <i>A</i> <sub>2</sub>	<i>HQ</i>	-	1	1 - <i>s</i>
<i>A</i> <sub>2</sub> <i>A</i> <sub>2</sub> × <i>A</i> <sub>1</sub> <i>A</i> <sub>1</sub>	<i>PQ</i>	-	1	-
<i>A</i> <sub>2</sub> <i>A</i> <sub>2</sub> × <i>A</i> <sub>1</sub> <i>A</i> <sub>2</sub>	<i>HQ</i>	-	1	1
<i>A</i> <sub>2</sub> <i>A</i> <sub>2</sub> × <i>A</i> <sub>2</sub> <i>A</i> <sub>2</sub>	<i>Q</i> <sup>2</sup>	-	-	1
		$\frac{p(p - sH/2)}{1 - sH/2}$	$\frac{2pq}{1 - sH/2}$	$\frac{q(q - sH/2)}{1 - sH/2}$

A bi-allelic selection model to describe observations of segregation for genes *HLA-A* and *HLA-B* in South Amerindians.

zygotes<sup>2</sup>. The maximum excess of heterozygotes over Hardy–Weinberg proportions (the most negative *F* value) occurs when the allelic frequencies are equal. For example, when the allelic frequencies are equal and *s* = 0.5, then  $\bar{w} = 0.854$  and *F* = -0.172.

Heterozygous females have lower fitness than homozygous females because of the lowered average fitness of their offspring. However, they appear to compensate precisely for this by the relatively higher fitness of offspring that are exactly like themselves (heterozygotes), thus explaining the neutrality equilibrium. The relatively higher fitness of their heterozygous offspring results in an overall excess of heterozygous offspring.

However, given two alleles in neutral equilibrium and the generation of a third allele by mutation, this new allele has a selective advantage. In other words, for situations with more than two alleles, such as most MHC genes, this model predicts that selection will maintain a stable polymorphism for multiple alleles.

This bi-allelic selection model has no effect on the dynamics of genetic change, but when the two alleles have equal frequency the model results in a minimum fitness and a maximum excess of heterozygotes. This is, to my knowledge, the first description of a selection model with these unique properties.

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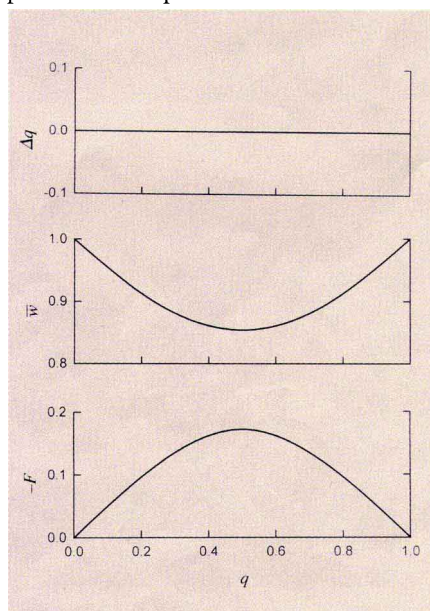
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## DNA answers the call of pipistrelle bat species

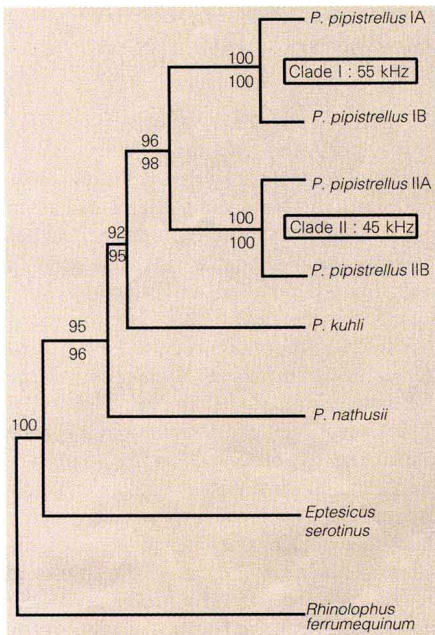
Groups of organisms that have been described as a single taxonomic unit on the basis of quantitative characters are increasingly proving to require more complex classification, when their evolutionary history is studied with molecular markers<sup>1</sup>. Here we report an analysis of mitochondrial DNA sequences from the two echolocating types<sup>2</sup> of Europe's most abundant and well-studied bat, the pipistrelle<sup>3</sup> (*Pipistrellus pipistrellus*). We describe genetic divergence that supports its reclassification as two different species.

Species-specific acoustic signals are especially important in nocturnal animals because of constraints on visual communication in the dark. Species can therefore often be identified by differences in their calls or other acoustic signals<sup>4,5</sup> when morphology and behaviour are poor discriminative tools<sup>6</sup>. A bimodal distribution of echolocation call frequencies of the pipistrelle indicates that there may be a previously unrecognized taxonomic division<sup>2</sup>.

Pipistrelles emit echolocation calls with the frequency of most energy close to either 45 or 55 kHz, yet the two phonic types are



**Figure 1** From top to bottom, the change in allelic frequency ( $\Delta q$ ), the mean fitness ( $\bar{w}$ ) and the excess of heterozygotes ( $-F$ ) as a function of allelic frequency for the selection model when *s* = 0.5.

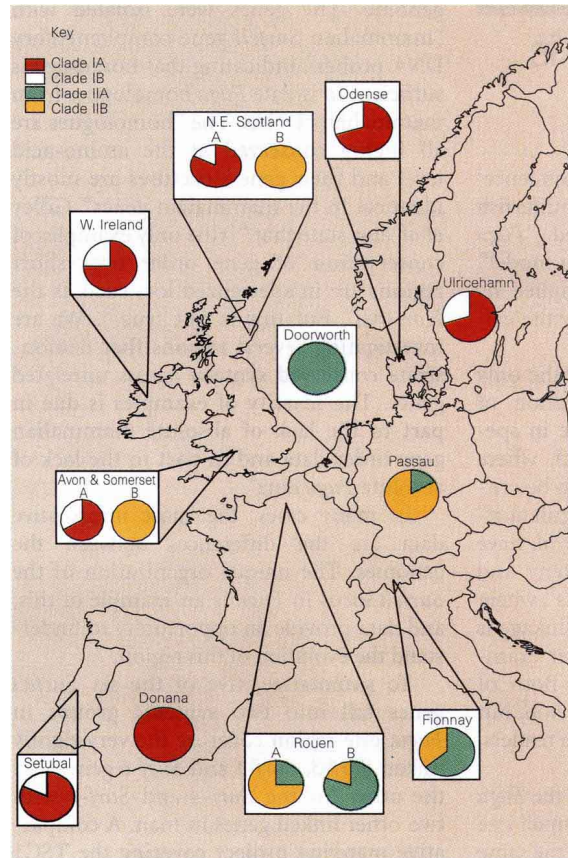


**Figure 1** Phylogenetic tree for 630 base pairs of cytochrome *b*, showing the relationships between haplotypes and phonic types in *Pipistrellus pipistrellus* and related bat species (*P. nathusii*, *n*=2; all other genotypes, *n*=4). The tree represents the single topology found using maximum-likelihood, maximum-parsimony and neighbour-joining methods in PHYLIP<sup>13</sup>. Numbers indicate bootstrap values: above the branches from a neighbour-joining analysis, below the branches from a parsimony bootstrap analysis (1,000 replicates each). The separation of the two phonic types is fully supported, with a sequence divergence of 11%. There is up to 95% support for the separation of *P. pipistrellus* from the other species.

indistinguishable in multivariate analyses of flight morphology<sup>2</sup>. Maternity colonies contain bats of only one phonic type, but the distributions of the two phonic types overlap for much of their European range<sup>2</sup>.

We compared 630 base pairs of aligned cytochrome *b* sequences from four bats of each phonic group and other related species. This revealed four haplotypes which clustered into two distinct clades. Within each clade (termed I and II) the haplotypes showed a sequence divergence of less than 1%, whereas between clades the divergence exceeded 11% (Fig. 1) (NCBI accession numbers U95499–U95514). Divergence of this magnitude is similar to that found between reproductively and morphologically distinct bat species<sup>7,8</sup>. Comparing the differences with data from the vertebrate molecular clock<sup>9</sup>, the phonic types of *P. pipistrellus* seem to have diverged 5–10 million years ago, and so could be considered as distinct phylogenetic species<sup>10</sup>.

Individuals from each maternity roost were of a single phonic type, which was associated with sequences from the same cytochrome *b* clade, but the abundance of



**Figure 2** Distribution of mitochondrial DNA genotypes and phonic types of pipistrelle in Europe. Each pie chart represents one maternity roost. The colonies, with number of bats sequenced, echolocation call frequencies where available (and number of bats recorded) are: north-east Scotland (A) 12 sequenced; (B) 10 sequenced, 45.6±1.3 kHz (12 recorded); Avon and Somerset, UK (A) 15, 57.6±1.6 kHz (50); (B) 8, 46.1±1.5 kHz (39); Ireland (A) 8; Rouen, France (A) 8; (B) 5; Setubal, Portugal 11; Odense, Denmark 10; Ulricehamn, Sweden 23, 53.6±1.8 kHz (58); Passau, Germany 11; Fionnay, Switzerland 11; Doorworth, Holland 14; Donana, Spain 8.

the two haplotypes within each clade varied geographically (Fig. 2). In clade I (55 kHz), haplotype IB was always rarer. In clade II (45 kHz), British bats always belonged to haplotype IIB whereas roosts on mainland Europe often contained both haplotypes. The distributions of the two clades overlapped for much of Britain and mainland Europe, although clade I bats tended to occur near the edge of the pipistrelle's range (Ireland, Sweden and Portugal).

It is more likely that acoustic divergence between species occurs after genetic isolation, rather than functioning as a mechanism to promote speciation<sup>11</sup>. In most animals (for example, grasshoppers, crickets, birds, frogs and toads) vocal divergence between species seems to be driven by female choice through sexual selection<sup>12</sup>. For the echolocation calls of bats, however, natural selection seems to have shaped call design so that optimal echoes can be received from targets of different sizes. Bat echolocation signals may be more constrained than are mating songs of other animals, and are unlikely to become more elaborate as a result of sexual selection.

Our finding that one of Europe's most widespread, abundant and well-studied mammals exists as two cryptic species has implications for the study of bat biodiversity. If a mammal that has received considerable scientific attention has only now been shown to exist as two species, many of the over 900 bat species whose echolocation calls and genetics have yet to be studied may

also comprise groups of morphologically similar but evolutionarily distinct species.

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