Single-gene speciation by left–right reversal

A land-snail species of polyphyletic origin results from chirality constraints on mating.

A single gene gives rise to the mirror-image form of a snail's body plan, which could become established as a different species if mating is prevented between snails of different chirality by genital mismatch[1–3]. Here we use molecular phylogeny to demonstrate the parallel evolution of reversal between left and right lineages of the Japanese land snail *Euhadra*. We find that the different mirror-image forms have evolved in favour of the genetically dominant handedness as a result of single-gene speciation.

Speciation accompanying the left–right reversal of the entire ontogeny (chiral speciation) is unique to snails and can be visualized by the coiling direction of their shells[4] (Fig. 1a). The chirality (or 'handedness' — occurring as 'sinister' and 'dexter' forms) of snails is determined by the maternal nuclear genotype at a single locus[5]. Because of the physical difficulty of two-way copulation between snails that have opposite coils (Fig. 1b), frequency-dependent selection occurs to eliminate the chiral minority[3,6–8] and antagonize chiral speciation. Offspring from each mother all develop with an identical chiral phenotype, regardless of their genotype. This peculiar mode of inheritance could be advantageous in enabling the dominant reversal allele to proliferate in small, isolated populations[2,9]. Once the mirror-image variant exceeds 50%, selection fixes the reversal. Reproductive isolation by handedness would then be complete.

In *Euhadra*, 4 of 20 species are sinistral. We discovered three notable processes of chiral evolution, based on analysis of mitochondrial DNA phylogeny (see supplementary information). First, the sinistral taxa are all derived from a single sinistral ancestor. Second, reversal to a dextral species from the sinistral ancestor has occurred in at least three independent lineages. Third, all of the haplotypes of a dexter *E. aomoriensis* are included within a sinister *E. quaesita*.

This pattern cannot be explained by introgression of mitochondrial DNA between *E. quaesita* and other dextral species, because opposite handedness prevents hybridization. Both mitochondrial-DNA phylogeny and a similar shell morphology (Fig. 1a) indicate that *E. aomoriensis* is derived from *E. queasita*. *E. aomoriensis* is not merely a dextral morph of *E. queasita*, as the two differ in shell sculpture although they are partly sympatric. We conclude that *E. aomoriensis* has speciated from *E. quaesita* by virtue of its chirality. The two species mutually failed in interchiral copulation despite frequent attempts, as did the chiral variants of *Bradybaena similaris*[3] (see supplementary information). Chiral reversal is therefore an acute pre-mating mechanism of isolation.

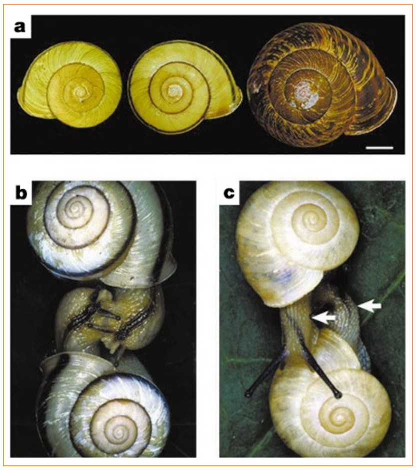

Figure 1 Mirror-image species and copulation in snails. **a,** Shells of *Euhadra*: left, *E. quaesita* (sinistral, or left-handed); middle, *E. aomoriensis* (dextral, or right-handed); right, *E. senckenbergiana* (dextral). Scale bar, 10 mm. The remarkable similarity of the shells reflects the chiral speciation of *E. aomoriensis* from *E. quaesita*, although *E. aomoriensis* has traditionally been considered to be a subspecies of *E. senckenbergiana* (see text). **b,** Successful two-way copulation between dexters of *E. congenita*. **c,** Genital mismatch between a sinistral variant (top) and an ordinary dexter (bottom) of *Bradybaena similaris*. Arrows indicate genital openings that cannot be joined.

Reversal to a dextral species has occurred multiple times after only sinistral evolution, so chiral speciation must have been easier to the dexter than to the sinister. We tested chiral inheritance in the family Bradybaenidae, which includes *Euhadra*, using a single dextral adult *Bradybaena similaris* collected with an exceptional sinistral variant[3]. The dexter, which presumably had been inseminated by multiple conspecific dexters, produced sinistral offspring.

According to the single-locus, delayed-inheritance model with dextral dominance, the dextral mother would have been a recessive homozygote and the sinistral offspring (F_1) would have been heterozygotes. Seven pairs of F_1 sinisters produced 131 dexters (F_2), in which only 30 produced sinistral offspring and others did not. The 30 dexters must therefore have been recessive homozygotes. The ratio of 30 to 101 is consistent with a prediction of allelic segregation at a single locus, with dextral dominance in F_2 ($\chi^2 = 3.01$, $P = 0.62$). The repeated twofold reversals to dextral species must therefore reflect the greater chance of fixation for the dominant allele, as predicted by theory[9,10].

The polyphyly of *E. aomoriensis* in mitochondrial-DNA phylogeny could have resulted from parallel evolution of *E. aomoriensis*, or from introgression or ancestral polymorphism of mitochondrial DNA. As the parallel evolution of three dextral lineages has occurred, multiple peripheral populations of *E. quaesita* may also have fixed the dextral allele independently. The polyphyly of ancestral species is expected where prompt speciation recurs in peripheral isolates[11]. Incipient single-gene speciation without genome-wide changes must allow *E. aomoriensis* to interbreed with polyphyletic descendants. *E. aomoriensis* may therefore be a unique example of a polyphyletic animal species.

Introgression is impossible unless the reversal allele has persisted in either species under stringent selection. Any introgression must therefore have been limited to a short time before chiral fixation. Ancestral polymorphism would not easily survive chiral speciation in small, isolated populations.

Left–right reversal in reciprocally mating snails can accomplish pre-mating isolation at a single locus, overcoming the majority rule of handedness. Our results indicate that single-gene speciation is possible, at least in hermaphroditic snails, contrary to the traditionally held view[2,12,13].

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