Internet 'shellebrity' reflects on origin of rare mirror-image snails

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While animal bodies are typically bilaterally symmetric on the outside, the internal organs nearly always show an invariant left-right (LR) asymmetry. In comparison, snails are both internally and externally LR asymmetric, outwardly obvious in the shell coiling direction, or chirality. Although some species of snail are naturally variable for chirality, sinistral individuals occur very rarely in most species. The developmental and genetic basis of these rare mirror-imaged individuals remains mysterious. To resolve this issue, the finding of a ‘one in a million’ sinistral garden snail called ‘Jeremy’ was used to recruit citizen scientists to find further sinistral snails. These snails were then bred together to understand whether their occurrence is due an inherited condition. The combined evidence shows that rare sinistral garden snails are not usually produced due to a major effect maternal Mendelian locus. Instead, they are likely mainly produced by a developmental accident. This finding has relevance to understanding the common factors that define cellular and organismal LR asymmetry, and the origin of rare reversed individuals in other animal groups that exhibit nearly invariant LR asymmetry.

1. Introduction

There is an emerging consensus that the left-right (LR) asymmetry of the main body axes likely originates from the cytoskeletal dynamics that underlie the asymmetric behaviour of individual cells [1-3]. In this respect, snails are an important part of understanding LR asymmetry because they are the only animal group for which early development may ordinarily produce forms that are wholly mirror-imaged in their body asymmetry [4, 5]. Moreover, as the same set of genes define [2] and amplify [6] LR asymmetry in snails and other animals such as vertebrates, the natural genetic variation in snail chirality has the potential to be a valuable resource in understanding the evolution and development of LR asymmetry, including the invariant asymmetry of other animal groups [4].

In snail species in which both mirror-image versions are relatively common, natural and sexual selection have been implicated in promoting and maintaining the evolution of different chiral forms [7-11]. A stereotypic alternating spiral cleavage early in development ultimately produces snails with clockwise or anticlockwise coiling shells, with mirror-image bodies to match (figure 1).

To understand this variation, nearly one hundred years ago Boycott and Diver, and Sturtevant showed that chiral variation in pond snails is controlled by a maternal effect gene [12-14]. A dominant maternal *D* allele in *Lymnaea* (*Radix*) *peregra* determines a clockwise or dextral twist in embryos during the third cleavage. Subsequently, the same broad pattern of inheritance has been shown in all species studied, although the sinistral allele is sometimes dominant [refs in 15]. Different versions of a formin gene determine the variation in LR asymmetry in the pond snail, *Lymnaea stagnalis* [2, 16-18], and a formin is also associated with variation in a land snail, *Bradybaena similaris* [19].

One significant remaining issue is that the mutations that make reversed snails are pathological in the most studied species [20-22]. The genes that enable snails to ordinarily vary in their chirality, without pathology, are therefore still wholly unknown [4]. Another puzzle is that in the majority of snail species, mirror-imaged individuals are rare – as in other animals – to the extent that ‘lefties’ are cherished by shell-collectors. The origins of these sinistral individuals are not known and have been investigated only rarely.

In trying to understand the origins of rare sinistrals, it is difficult to test the inheritance of chirality. Lefty snails do not usually mate with normal snails because the genitals are on the opposite side of the head [23]. The courting behaviour is also chiral [24, 25], and individuals in most species prefer to outcross, so self-fertilisation is also not an option. A further problem is that the few published studies on the inheritance of chirality in rare sinistrals are anedoctal [26], or only considered the chirality of the first generation of offspring [27]. The latter is an issue because if sinistrality is due to a recessive allele, then up to three generations of breeding are required to bring together homozygous sinistral alleles in the same individual (figure 2).

Following the discovery of ‘one in a million’ sinistral garden snail *Cornu aspersum* in South West London, I (A.D.) launched a publicity #snaillove campaign to find a mate. Initially, I hoped to communicate our previous work on the science of LR asymmetry [2], which had been well received in the scientific press [28, 29], but featured less in the wider media. However, by bringing together a group of citizen scientists [30], it offered an opportunity to understand the genetics of chirality in a species in which sinistrals are very rare.

The snail that we named ‘Jeremy’ (figure 1, named after the garden-loving, left-wing UK politician Jeremy Corbyn) became an internet ‘shellebrity’ and media sensation, featured in more than 1000 news [e.g. 31, 32] and science articles [33, 34] over the course of several years. Ironically, the same scientific advances [2] that had only been of limited wider interest some months earlier, were headline news when introduced by a lonely snail.

There was also a happy ending for Jeremy, the snail. A snail enthusiast and a snail farmer/restauranteur each found lefty snails (figure 1). One of these snails mated with Jeremy, producing offspring just before the snail died [31]. The work here describes the outcome of the research that was enabled by the finding of this rare snail.

2. Methods

(a) The culture of snails.

Snails were fed a diet composed of ground grass pellets, porridge oats and chalk, as described previously [35], supplemented with loose-head lettuce. To enable egg-laying, individuals or pairs of individuals were housed in small plastic aquaria with a soil substrate.

(b) Breeding strategy – theory.

If a sinistral chirality mutation is dominant *S*, then the mother of a sinistral snail was most likely of genotype *Ss*. Sinistral F0 snails will be of genotype *Ss* or *ss* (figure 2a). F0 snails of genotype *Ss* will produce F1 offspring of sinistral phenotype, but no generation of offspring from individuals of genotype *ss* will produce sinistral offspring.

Alternatively, assuming a rare sinistral snail is likely produced by mutation in a recessive maternal effect gene, *d*, the mother must have had genotype *dd*. Therefore, sinistral F0 snails will likely have a genotype *Dd*, and produce dextral offspring. The best case scenario is that the same chirality locus is mutated in both F0 parents (figure 2b). In that circumstance, approximately 1/4 of the F1s will be of homozygous sinistral *dd* genotype, and so produce sinistral offspring. However, if different loci are involved in causing sinistrality in different F0 individuals (figure 2c), then ~1/2 of the F1 offspring would be heterozygous *Dd* and half homozygous *DD,* for each locus. All will produce dextral offspring. In that circumstance, it is necessary to sib-mate F1 snails, raise the F2 generation and score the chirality of the F3 snails, to infer the chirality genotype of the F2 mother. The expectation is that ~1/4 of the F2 snails will produce sinistral offspring.

As garden snails are simultaneous hermaphrodites, but have a strong preference for outcrossing, the above theory assumes that self-fertilisation is rare. Occasional self-fertilisation would increase the chances of fixation of a rare sinistral chirality allele.

(c) Breeding strategy – crosses.

To understand the possible inheritance of sinistrality in garden snails, a three generation mating programme was undertaken, necessary in case the phenotype was due to a recessive maternal effect allele (figure 2; see above). To achieve this, sinistral garden snails were brought to the laboratory at Nottingham and bred; these individuals were assumed to be virgin, because sinistral snails are not usually able to mate with dextral snails. In most cases, mating pairs of snails were generally kept together until one of the snails laid eggs; the two adult snails were then removed without knowing which was the mother. In a few cases, individual mothers were mated sequentially to more than one snail (e.g. C3 x C2/C1), and fathers removed. The offspring were from a known mother, but likely of mixed paternity.

The sinistral F0 snails were crossed with each other, in the order in which they were received, and then the chirality of sets of F1 offspring was scored, to infer the chirality genotype of the mother. A proportion of these F1 offspring were then raised to adulthood, and sib-mated to create individuals that could be homozygous for a potential chirality locus. Again, the chirality of sets of F2 offspring was scored, to infer the chirality genotype of the F1 mothers. Then, a proportion of the F2 offspring were raised to adulthood, and again sib-mated. Finally, the chirality of the F3 offspring from the F2 parents was then scored, to infer the chirality genotype of the F2 mother.

Separately, I also received information on the chirality of the F1 offspring from three sinistral garden snails in other locations. A series of crosses were also undertaken in France by P.T., involving raising and crossing the dextral F1 offspring of sinistral snails from a farm, from which an F2 generation was produced.

Data were also gathered from snails in a related genus, *Cepaea*, because the general publicity meant that we also received three sinistral individuals in this otherwise dextral genus.

3. Results

Altogether, the publicity helped me gain records of 45 sinistral garden snails, including 34 from snail farms, alongside three sinistral grove snails *Cepaea nemoralis* and one white-lipped snail *C. hortensis* (table S1).

The records enable an estimate of the frequency of sinistrals in garden snails. For example, one Spanish farm produced four, three and two snails over three consecutive years (2017-2019); one sinistral was found in 2018 and four in 2019 in a Polish snail farm; a farm in Mallorca recorded just one sinistral in 2016. Taking into account the total production of these farms, this gives an estimate for sinistrals of about 1 in ~40,000. Although this might suggest that the frequency of sinistrals is considerably higher than the 'one in a million' put to the public, this is an upper bound because it does not account for occasions in which no sinistrals were found.

To understand the possible inheritance of sinistrality in garden snails, a three generation mating programme was undertaken, necessary in case the phenotype was due to a recessive maternal effect allele (figure 1). None of the original sinistral snails (F0 generation) produced sinistral offspring (13 snails, 1120 offspring; table 1, S1). Three other individuals in two species of *Cepaea* also produced only dextral offspring (table S1). None of 63 F1 snails, co-derived from 9 different sinistral F0 mothers, produced sinistral offspring (3598 offspring). The only exception was a cross involving dextral F1 offspring in France, 6 snails from 32 of which produced mixed broods, containing 17 sinistral offspring total in 6302 offspring (table 1, S2). Of broods that were mixed, 1.7% of the individuals were sinistral. This result is consistent with the presence of a partially penetrant recessive sinistral allele in each of the F0 parents (26:6 consistent with 3:1 expectation in F1 offspring, $X^2 = 0.09$, p-value = 0.8). Finally, none of 107 F2 snails, co-derived from 5 different sinistral F0 mothers, produced sinistral F3 offspring (3576 offspring; table 1, S3).

4. Discussion

The combined evidence shows that rare sinistral garden snails are not produced due to a major effect maternal Mendelian locus, as in most other snails that have been studied [36-39], including the most well known [12, 13]. Instead, a better explanation is that sinistrals are sometimes produced by developmental accident, perhaps due to a chance, or environmentally-induced, reversal, in the third cleavage [40]. This reversal may happen more frequently in the presence of an allele which partially interferes with the third cleavage – as in the French snails – so producing a few sinistral offspring in an otherwise dextral brood.

These findings are consistent with the few previous studies, which generally only considered the first generation of offspring. For example, in *Helix pomatia* several studies have shown that the F1 offspring from sinistral crosses are dextral ([page 641 in ref 27]), but further generations were not considered. In *C. hortensis*, two sinistrals were mated in the 1970s, and one of them produced 64 dextral offspring. At the time, the authors concluded that sinistrality in *C. hortensis* was unlikely to be genetic in origin [41]. However, following the publicity for 'Jeremy', one of the authors communicated with me. He had continued to breed from the snails. In the next generation, there were 4 sinistrals out of ~2000 offspring, which perhaps implies some genetic contribution to chiral variation in this species (Michael Ratsey pers. comm.).

As noted for a wide range of other animals [42], chiral variation in snails is likely due to environment, chance or genetic factors, all of which may occur in the same taxonomic group of snails. The ‘helicoid’ superfamily of land snails [43] may be particularly useful for further study because it contains species that do not ordinarily vary, such as the garden snail, as well as others that show inherited chiral variation [9]. In some species, including *Bradybaena* in the helicoid group, the mutant allele partly randomises the direction of the spiral cleavage, so that mixed broods are regularly produced [19, 39, 44]. However, in *Bradybaena* the mutation apparently impairs the expression of a formin gene, so that a proportion of the embryos fail to develop [19]. The same pathology phenotype and mixed broods also occur in two species of pond snails [20, 22, 36, 45]. Mixed broods are occur occasionally in *Partula* [44], alongside differences in shell shape in some species [46-49]. In garden snails, two snails produced mixed broods (table 1), but there are insufficient data to determine if pathology is implicated.

In comparison, in *Euhadra* snails, also in the helicoid group, there is no evidence of pathology and the widespread occurrence of both dextral and sinistral forms in different regions of Japan. It has been argued previously that these different forms are reproductively isolated because of difficulties in mating, and so undergo ‘single-gene speciation’ [11]. Both theory and empirical evidence suggest ongoing gene-flow, so that chiral change can only be viewed as one step towards speciation [9, 10]. A final exceptional genus in the helicoid group are *Amphidromus* snails, in which snails may have increased mating success with individuals of the opposite chirality [8, 50], potentially resulting in negative frequency dependent selection.

In the French garden snails, it should be possible to map and then identify the gene/allele that is responsible for producing sinistrals. The mutation is likely due to a change in gene expression that alters the topology of the cytoskeleton and the subsequent chiral twist at third cleavage [4]. Although this will be an interesting finding, of relevance to the wider field of LR asymmetry, the more important task should be to identify the mutations and the genes that enable mirror-image versions of some snail species to exist without pathology in wild populations.

In the future, understanding the evolution and development of the chiral twist in snails may be key in revealing the common factors that define cellular and organismal LR asymmetry, and the almost absolute invariance of whole-body LR asymmetry in other animal groups. This may also contribute to understanding the variable asymmetry that some animals have in later development [42, 51].

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Table 1. Summary of the sinistral snails used, the chirality of their offspring, and the chirality of the offspring in subsequent generations. Where two potential fathers are listed, the offspring probably represent a mixed brood.

F0 snails					**F0 crosses**			F0 offspring chirality		**F1 snails**	F1 offspring chirality		**F2 snails**	F2 offspring chirality	
ID	Name	Finder	Country	Source	Mother		Father	Dextral	Sinistral	No. mothers	Dextral	Sinistral	No. mothers	Dextral	Sinistral
C1	Jeremy	David Reid	UK	wild											
C2	Lefty	Jade Sanchez Melton	UK	wild	C2	x	C3	46		5	160		8	195	
C3	Tomeu	Miguel Angel Salom	Spain	farm	C3	x	C2	279		3	77		27	836	
					C3	x	C2/C1	56		12	741				
C4	Senda	Aurora Heras	Spain	farm	C4		self?	2		3	127				
C6	Jara	Aurora Heras	Spain	farm	C6		self?	6		3	214		15	470	
					C6	x	C7	79		10	446		23	733	
C7	Indi	Aurora Heras	Spain	farm	C7	x	C6	63		11	651		20	677	
C11	CRASH	Álvaro Puente Cano	Spain	wild	C11	x	C4	79		7	334		14	665	
C82	Melchor	Aurora Heras	Spain	farm	C82	x	C83/C84	67							
C83	Gaspar	Aurora Heras	Spain	farm	C83	x	C82/C84	102							
C84	Baltasar	Aurora Heras	Spain	farm	C84	x	C82/C83	53							
A		Philipe Thomas	France	home	A	x	B	51		4	324				
B		Philipe Thomas	France	home	B	x	A	119		3	320				
S1		Mike Vergnes	France	farm	S1	x	S2	35		10	2156	14			
S2		Mike Vergnes	France	farm	S2	x	S1	83		22	4129	3			
JXD		Jiří Doležal	Ireland?	farm	JXD		not known	n/a		2	204				

Figure 1. Sinistral and dextral garden snails, *Cornu aspersum*. Top: The image that launched the media campaign. The shell of the uppermost snail, 'Jeremy', coils anti-clockwise, whereas the bottom snail, 'Theresa', has a shell that coils clockwise. Bottom: Four sinistral snails from Spanish snail farms, alongside Jeremy second right.

Figure 2. Breeding strategy. (a) Assuming a sinistral allele is dominant, half of sinistral F0 parents will produce sinistral offspring: sinistral offspring (shaded box) will be produced from *Ss* mothers. (b) Assuming sinistral allele is recessive and the *same* causal locus in both parent F0 snails: one in four of the offspring from F1 snails will have dextral shells, but a homozygous sinistral genotype, *dd*. These snails will produce sinistral F2 offspring (shaded box). (c) Assuming sinistral allele is recessive and a *different* causal locus in both parent F0 snails: a further round of sib-mating is required to produce homozygous sinistral offspring. Snail image reproduced from ref [4] with permission from Elsevier.