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

Angus Davison¹, Philippe Thomas² and 'Jeremy the snail' citizen scientists

1. School of Life Sciences, University Park, University of Nottingham, NG7 2RD, UK.

2. 87 rue du Bois 62136 Richebourg, France.

Author for correspondence: Angus Davison e-mail angus.davison@nottingham.ac.uk

Supplementary Material

Explanatory note on authorship

In 2016 I (A.D.) received a sinistral garden snail *Cornu aspersum*, found by Dr David Reid, a retired malacologist from the Natural History Museum in London. To communicate the science of asymmetry and to understand the genetics, I launched a media campaign to find a mate for "Jeremy the snail", reaching a potential audience of 1.8 Billion persons, via 1000+ media articles and interviews [1]. The publicity was then used to assemble a group of persons, made up of individuals who fortuitously had found a sinistral snail in the wild, persons keeping snails as pets, persons associated with museums, and persons associated with snail farms. The project would not have been possible without the involvement of these persons/farms, and so in line with a recent recommendation [2] they are named as citizen science authors to this work.

One person, Philippe Thomas (Bethune, France; phthomas62@gmail.com) contributed more than others. He designed his own breeding programme, sourcing snails from a local snail farm belonging to Mike Vergnes (Escargots St Félicien, France; mikevergnes@yahoo.fr). He is therefore also listed as a co-author.

The other individuals were Jade Sanchez Melton (JSMelton@gmx.co.uk), Laura Sampson (lauraasimpson@sky.com), Peter Osborne (UK, peter.a.osborne@btinternet.com), Álvaro Puente Cano (Spain, apuentecano@hotmail.com), Philippe Thomas (France, phthomas62@gmail.com), Cassie Holmes-Brown (Australia, cassiejhb@outlook.com), Harold and Abby Lamb (Canada, harltoncres@hotmail.com), Susanne Sayici (Austria, s.sayici@yahoo.de).

Individuals associated with museums were Lindsey Groves/Alex Baristow (USA, Igroves@nhm.org, alexbairstow3@hotmail.com), Frank Wesselingh (Netherlands, frank.wesselingh@naturalis.nl).

Persons associated with snail farms were Miguel Angel Salom (Spain, miguel_angel_molas@hotmail.com), Aurora Heras (Spain, auroraheras47@gmail.com), Marcos Aguado (Spain, marcosaguado74@gmail.com), Jiří Doležal aka jxd (Czech Republic – information on Irish and Greek farms, jxd@jxd.cz), Victoria's snails/Filip Zasadowski (Poland, victoriassnail@gmail.com).

Results

F0 generation

All offspring were all dextral, involving 14 garden snail mothers and 3 *Cepaea* mothers. See table 1 for summary, and table S1 for fuller details.

1) C3 x C2. Dextral offspring were raised from both parents as mothers. 2) C3 x C1/C2. After C3 mated to C2, it was then mated to C1. Dextral offspring were then raised from C3 as mother, assuming mixed paternity. 3) C4. A few dextral offspring hatched from a possible self-fertilisation. 4) C4 x C11. Dextral offspring were raised from C11 as mother. 5) C6. A few dextral offspring hatched from a possible self-fertilisation. 6) C6 x C7. Dextral offspring were raised from both parents as mothers. 7) Data were also received from sinistral snails

crossed by Jiří Doležal; the dextral offspring were received in Nottingham. 8) Data were received from four sinistral snails crossed in France, all producing dextral offspring. 9) The dextral offspring of 3 sinistral *Cepaea* were also raised.

If the sinistral phenotype was an inherited condition, and the sinistral allele is dominant (figure 2a), then approximately half the F0 individuals should produce sinistral offspring. Given an expected 1:1 ratio, the observed ratio of 14:0 is unlikely due to chance ($X^2 = 6.9$, p-value = 0.009). There is therefore no evidence from the F0 generation that variation in chirality is an inherited in garden snails.

F1 generation

The F1 offspring were crossed with full siblings, with the exception of the offspring of the C3 \times C1/C2 cross, which were a mixture of full- or half-siblings. All offspring were dextral, with the exception of one cross. See table 1 for summary, and table S2 for fuller details.

1) 8 F1 offspring from C3 x C2 all produced dextral offspring (n=237). 2) 12 F1 offspring from C3 x C1/C2 also only produced dextral offspring (n=741). 3) 2 F1 offspring from a possible self-fertilisation of C4 produced dextral offspring (n=127). 4) 7 F1 offspring from C11 x C4 produced dextral offspring (n=334). 5) 2 F1 offspring from a possible C6 self-fertilisation produced dextral offspring (n=214). 6) 18 F1 offspring from C6 x C7 produced dextral offspring from sinistral snails crossed by Jiří Doležal produced dextral offspring (n=204).

Separately, 7 F1 offspring from 2 sinistral mothers (A x B; table S2) raised by Philippe Thomas produced dextral offspring (n=644). Subsequently, he received a further batch of dextral offspring from two further sinistral snails (S1 x S2). 32 were raised to adulthood, of which 6 produced 17 sinistral offspring in otherwise dextral broods (table S2).

If the sinistral phenotype was due to the same recessive condition in all snails, then the expectation is for 1/4 of the F1 offspring to produce sinistral offspring (figure 2b). The observed ratio was 85:6 ($X^2 = 10.2$, p-value = 0.001). An alternative hypothesis is that different loci were mutated in different F0 mothers, in which case the expectation is that 1/4 of the F1 offspring in each of the maternal lines might have sinistral offspring. There were insufficient observations to test this hypothesis for most maternal lines, with the exception of the F1 individuals derived from the sinistral French cross S1 x S2. For these, the result is consistent with the presence of a partially penetrant recessive sinistral allele in each of the F0 parents (26:6; $X^2 = 0.09$, p-value = 0.8).

F2 generation

The F2 offspring were crossed with full siblings. All offspring were dextral. See table 1 for summary, and table S3 for fuller details.

1) 19 F2 offspring from C3 x C2 all produced dextral offspring (n=468). 2) 16 F2 offspring from C3 x C1/C2 also only produced dextral offspring (n=563). 3) 14 F2 offspring from C11 x C4 produced dextral offspring (n=665). 4) 15 F2 offspring from a possible C6 self-fertilisation produced dextral offspring (n=470). 5) 43 F2 offspring from C6 x C7 produced dextral offspring (n=1410).

This result is consistent with a developmental accident as an explanation. An alternative is that the sinistral phenotype was a recessive inherited condition, with different loci involved in different F0 mothers. Unfortunately, it is not possible to know which F2 snails might have arisen from F1 *Dd* x *Dd* crosses, especially if it is assumed that only some of the sinistrals were produced due to an inherited condition. Overall, 107 F2 snails were derived from 5 different F0 mothers. Assuming a possible independent mutation in each of these maternal lines, then the expectation is that ~1/4 of them may have been the correct type. The

observed ratios were 27:0 (C3; $X^2 = 5.6$, p-value = 0.018), 8:0 (C2; $X^2 = 0.6$, p-value = 0.5), 38:0 (C6; $X^2 = 8.7$, p-value = 0.003), 20:0 (C7; $X^2 = 3.7$, p-value = 0.06), 14:0 (C11; $X^2 = 2.0$, p-value = 0.2).

References

- 1. Anonymous. 2017 Jeremy the Snail, Media Peformance report, 1 Oct 2016 30 Oct 2017. (University of Nottingham / Meltwater.
- 2. Ward-Fear, G., Pauly, G.B., Vendetti, J.E. & Shine, R. 2020 Authorship Protocols Must Change to Credit Citizen Scientists. *Trends Ecol Evol.* (doi:10.1016/j.tree.2019.10.007).