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Biol. Lett. 2009 **5**, 20-22
doi: 10.1098/rsbl.2008.0528

References

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Mating behaviour in *Lymnaea stagnalis* pond snails is a maternally inherited, lateralized trait

Angus Davison*, Hayley T. Frend, Camile Moray, Hannah Wheatley, Laura J. Searle and Markus P. Eichhorn

School of Biology, University of Nottingham, Nottingham NG7 2UH, UK

*Author for correspondence (angus.davison@nottingham.ac.uk).

Lateralization of the brain has traditionally been considered a specialization that is confined to the vertebrates, but recent studies have revealed that a range of invertebrates also have a brain that is structurally asymmetric and/or each side performs a different set of functions. Here, we show that the precopulatory mating behaviour of the pond snail *Lymnaea stagnalis* is lateralized. We present evidence that the asymmetry of the behaviour corresponds to the sinistral or dextral shell coil, or chirality, of the snail, and is apparently also controlled by a maternal effect locus. As sinistral snails also tend to have mirror image brains, these findings suggest that the lateralized sexual behaviour of *L. stagnalis* is set up early in development, and is a direct consequence of the asymmetry of the entire body.

Keywords: chirality; lateralization; maternal effect; Mollusca

1. INTRODUCTION

The lateralization of the brain has for several decades been a research topic that was confined first to humans, and subsequently expanded to include other vertebrates (reviewed in Rogers 2002). By contrast, the functional asymmetry of invertebrate brains has been relatively ignored, although it is now becoming clear that the phenomenon is probably widespread. For instance, in the honeybee *Apis mellifera*, there is a laterality in responding to learnt odours, in that bees respond better when they are trained through their right antenna (Letzkus *et al.* 2006). Separately, it has been shown that 1–2 hours after training, recall is possible mainly when the bee uses its right antenna, but by 6 hours a lateral shift has taken place, so the memory is recalled when the left antenna is in use (Rogers & Vallortigara 2008). As lateralized behaviour has also been found in *Drosophila*, *Octopus*, a spider and a water bug (Ades & Ramires 2002; Byrne *et al.* 2002; Pascual *et al.* 2004; Kight *et al.* 2008), the implication is that there has been parallel evolution of lateralization, and also that the effective functioning of memory formation in animals, whether vertebrate or invertebrate, requires a lateralization of processing (Rogers & Vallortigara 2008).

A key problem in understanding lateralization is a general lack of direct evidence for inherited phenotypic variation, as well as a poor understanding of the selective forces that drive lateralization (Vallortigara & Rogers 2005). Thus, while there are often considerable differences in individual preferences for one side or another, the underlying genetic basis has rarely been characterized (exceptions include Barth *et al.* (2005) and Sagasti (2007)). By far, the majority of genetic models have been aimed at human studies. For example, a convincing case has been made that human handedness is inherited (McManus 1991; Klar 2003), but the problem in trying to map the gene(s) is that a hypothetical random factor adds noise to the system. Fish are perhaps the most promising non-human vertebrate model for understanding the genetics of laterality traits (Bisazza *et al.* 2000; Barth *et al.* 2005; Brown *et al.* 2007). In zebrafish, the *fsi* locus is associated with the inversion of both viscera and a subset of behavioural asymmetries, indicating a connection, albeit one that is complicated and not yet fully understood.

Set against this background, we investigated the occurrence and inheritance of a potential laterality trait in the pond snail *Lymnaea stagnalis*. The key issue was to understand if laterality traits in the pond snail are associated with both body chirality and brain asymmetry. Although this snail is a fully self-fertile hermaphrodite, it ordinarily outcrosses, and in any single mating, an individual takes the male role or the female role (Van Duivenboden & Ter Maat 1988). It has been reported previously that with dextral *L. stagnalis*, the individual playing the male role first climbs onto the shell of the female, circles over the shell in a *anticlockwise* direction until the female gonophore is reached (Van Duivenboden & Ter Maat 1988). There is, therefore, prior qualitative evidence of a chiral behaviour during mating, making it a good candidate for investigating a laterality trait.

2. MATERIAL AND METHODS

The sinistral stock of *L. stagnalis* is ultimately derived from the stock of Asami *et al.* (2008). The dextral stock was taken from a pond in the University in 2005, undergoing approximately five generations in the laboratory before the experiment began. Snails were kept in aerated 12 l plastic tanks and fed ad libitum with lettuce, changing the water about once per month. Subjects were tested for behavioural lateralization by placing virgin pairs together in small watch glasses, then observing their movements until the first stages of mating began. The direction of the circling was recorded. Each snail was used only once.

For a separate group of snails, the snail cerebral ganglia were dissected out so as to score morphological asymmetries in the brain. The shell was removed, and then the body was relaxed in 10 per cent Listerine for 10 min. The mid section, tentacles and mantle were pinned back. The skin was then snipped back from the entire head region to expose the oesophagus. This was cut, and pulled through to reveal the brain.

Chirality in snails is determined by a single locus with a maternal effect (Boycott & Diver 1923; Sturtevant 1923). This means that the phenotype of an individual is dependent upon the genotype of the mother. By convention, D is dextral allele and S is sinistral allele; dextral is dominant in *Lymnaea*. The dextral and sinistral stocks are genetically DD or SS, respectively (figure 1*a*). Virgin sinistrals and dextrals were mated, producing offspring that are genetically dextral (genotype=DS) but have a shell coil that is either sinistral (sinistral mother) or dextral (dextral mother) (F₁ generation in figure 1*a*). By allowing the sinistral F₁ mother to self-fertilize, offspring were produced with a dextral coil, but which are genetically DD, DS or SS (F₂ generation in figure 1*a*). Dextral SS individuals were identified by virtue of their producing sinistral young.

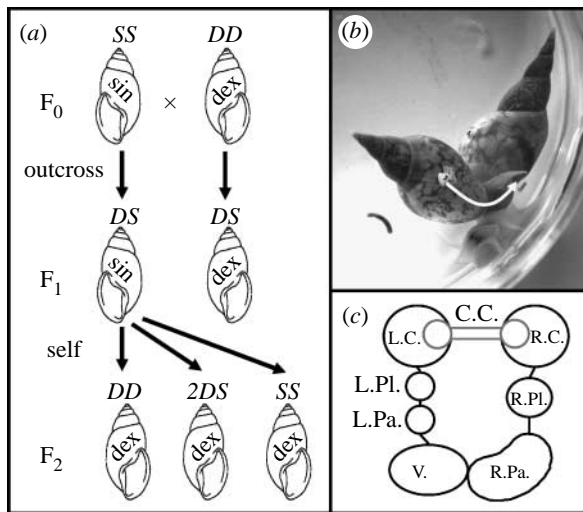


Figure 1. (a) A schematic of the crosses that were used to generate the snails with different combinations of coiling phenotype and genotype. (b) Anticlockwise circling behaviour of dextral male on dextral female, indicated by arrow. (c) A schematic of the asymmetric central nervous system (CNS) of a dextral *L. stagnalis*. L.C./R.C., left and right cerebral ganglia; L.Pl./R.Pl., left and right pleural ganglia; L.Pa./R.Pa., left and right parietal ganglia; V., visceral ganglion; C.C., cerebral commissure. Pedal and buccal ganglia are not shown.

3. RESULTS

The coiling direction of the shell of the snail playing the male role is a predictor of the direction of circling behaviour during mating and is independent of the chirality of the female. Of 46 dextral 'male' snails, we found that all circled in an anticlockwise manner (figure 1*b*), irrespective of whether they were paired with a dextral ($n=33$) or a sinistral snail ($n=13$) ($p<0.001$ using Fisher's exact probability test). Similarly, of 48 sinistral snails, all but one (possibly, an early mis-score) circled in a clockwise manner. Again, the circling direction of the sinistral male was independent of the chirality of the female (number of dextral females = 16; number of sinistral females = 32; $p<0.001$ using Fisher's exact probability test). The circling behaviour was entirely dependent on the maternal genotype, rather than the individual's own genotype. All 18 sinistral snails (genotype DS) and seven dextral snails (also genotype DS) circled either clockwise or anticlockwise, respectively; all 18 dextral snails (genotype SS) circled anticlockwise. This implies that the behaviour is maternally inherited.

Chirality in mating behaviour is matched by an asymmetry in the brain. *Lymnaea stagnalis* has a ring of nine ganglia that form a central nervous system (CNS) around the oesophagus, with two more distant buccal ganglia on the buccal mass (schematically shown in figure 1*c*, Chase 2002). In 37 out of 38 dextral individuals, the right parietal ganglion was fused with the visceral ganglion, so that the left visceral ganglion was unpaired, as is normal. One individual showed the reverse asymmetry. By contrast, in 30 out of 30 sinistral individuals, the left parietal ganglion was formed by fusion with a visceral ganglion. The CNS in sinistral *L. stagnalis*, therefore, has an asymmetry that is reversed compared with dextral snails.

4. DISCUSSION

The precopulatory mating behaviour of *L. stagnalis* pond snails is lateralized, in that dextral snails circle anticlockwise and sinistral snails circle clockwise. This asymmetry of behaviour is matched by an asymmetry of the brain, since snails have a brain asymmetry that corresponds with their coiling asymmetry. As the coil of the shell is determined by the maternal chirality genotype, and the asymmetry of the behaviour is in accordance with this, then it is possible, but not proven, that the behaviour is determined by a linked locus. These findings also suggest that the lateralized behaviour of the snails is set up early in development and is a direct consequence of the asymmetry of the body. Snails that circle in the opposite direction are unlikely to mate successfully, because the species is usually fixed for a single coiling type, so there is strong stabilizing sexual selection on circling direction.

Although the findings are novel, they are not unexpected. After all, gastropod molluscs in general are a model for understanding the neural control of behaviour (Chase 2002). It is also widely known that the mating behaviour of many species of snails has a chirality (e.g. Lipton & Murray 1979), and in *Helix aspersa*, morphological and physiological results have demonstrated a right-sided bias in the mesocerebrum that correlates with the fact that sexual behaviour takes place almost entirely on the animal's right side (Chase 1986; Koene *et al.* 2000). Finally, descriptions or figures of molluscan brains frequently illustrate pronounced asymmetries (Fretter & Peake 1975). *Lymnaea stagnalis* should now be added to the growing list of invertebrates for which lateralized behaviour has been documented. Moreover, although snails in general have not previously been subject to a strict test of lateralization, it is almost certain that many other species have the same lateralization for sexual behaviour.

The relationship between the chirality of the shell and the behaviour has not been tested previously, to our knowledge. The reason is because the vast majority of species are fixed in their chirality, so genetic experiments are not possible. The conclusion is that if the maternal effect chirality locus is the ultimate determinant of chiral mating behaviour, then this is put into effect by asymmetries in the brain. This simple scheme is actually complicated because numerous genes must mediate between the controlling chirality locus gene product and the effector genes with the brain.

It is intriguing that we discovered a dextral individual with a typical 'sinistral' brain. Although we were unfortunately not able to determine the asymmetry in mating behaviour of this individual, the discrepancy is in line with studies in humans, where it has been found that a variable proportion of individuals have a brain asymmetry that is not in the direction that might be expected based on their behaviour (McManus 2002).

We thank Joris Koene for the generous gift of sinistral snails, and Takahiro Asami, Edmund Gittenberger and Gerhard Falkner for establishing the sinistral stock in the first place. Menno Schilthuis, an anonymous referee and the editor provided useful comments on the manuscript.

This work was funded by the University of Nottingham and summer studentship grants from the Nuffield Foundation (URB/35713) and Genetics Society to H.T.F. and C.M., respectively.

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