

Dextral and sinistral *Amphidromus inversus* (Gastropoda: Pulmonata: Camaenidae) produce dextral sperm

Menno Schilthuizen · Bertie-Joan van Heuven

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Abstract Coiling direction in pulmonate gastropods is determined by a single gene via a maternal effect, which causes cytoskeletal dynamics in the early embryo of dextral gastropods to be the mirror image of the same in sinistral ones. We note that pulmonate gastropod spermatids also go through a helical twisting during their maturation. Moreover, we suspect that the coiling direction of the helical elements of the spermatozoa may affect their behaviour in the female reproductive tract, giving rise to the possibility that sperm chirality plays a role in the maintenance of whole-body chiral dimorphism in the tropical arboreal gastropod *Amphidromus inversus* (Müller, 1774). For these reasons, we investigated whether there is a relationship between a gastropod's body chirality and the chirality of the spermatozoa it produces. We found that spermatozoa in *A. inversus* are always dextrally coiled, regardless of the coiling direction of the animal itself. However, a partial review of the literature on sperm morphology in the Pulmonata revealed that chiral dimorphism does exist in certain species, apparently without any relationship with the coiling direction of the body. Though our study shows that body and sperm chirality follows independent developmental pathways, it gives rise to several questions that may be relevant to the understanding of the chirality of spermatid ultrastructure and spermatozoan motility and sexual selection.

Keywords Chirality · Mollusca · Left–right asymmetry · Ultrastructural polarity · Maternal effect genes

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M. Schilthuizen (✉) · B.-J. van Heuven
Netherlands Centre for Biodiversity Naturalis, P.O. Box 9517,
2300 RA Leiden, The Netherlands
e-mail: menno.schilthuizen@ncbnaturalis.nl

Introduction

Left–right patterning and the direction of chirality are often considered to be among the few developmental systems that are taxonomically sufficiently widespread to be studied in a meaningfully comparative way in almost all Bilateria. Palmer (2004), for example, mapped the evolutionary transitions among symmetry, directional asymmetry (in which within a species only one of the two possible chiral states occurs) and anti-symmetry (in which within a species the direction of chirality is random) on phylogenies for a large number of animal taxa. He found that directional asymmetry, in which the direction of chirality is almost always genetically determined, evolves directly from bilateral symmetry as frequently as via an intermediate anti-symmetric state, in which the direction of chirality is almost always random, non-genetically determined.

The genes responsible for symmetry breaking have been identified in a few instances. In deuterostomes, *nodal* and its target gene *Pitx* are asymmetrically expressed in the embryo. In Mammalia, the exact manner in which developmental inequality among left and right are set up by the fluid dynamics of the gene products have been elucidated (Hirokawa et al. 2006). In Gastropoda, *nodal* and *Pitx* were recently shown to play an important role as well. In these animals which, due to torsion and coiling early in development, show very conspicuous whole-body asymmetry, *nodal* and *Pitx* are expressed asymmetrically in the early and late trochophore larva, respectively (Grande and Patel 2008).

However, it has been known for a long time that other asymmetry-determining genes are acting upstream in gastropods. Already in the early days of Mendelism, gastropod coiling direction was found to be determined by a single locus with delayed maternal inheritance (Boycott and Diver 1923; Sturtevant 1923). This means that the chirality

of all offspring are determined by the mother's genotype, with one allele (either for dextral or for sinistral) being dominant (Schilthuizen and Davison 2005). In *Lymnaea stagnalis* (Linnaeus, 1758), the effect of this—yet unidentified (Harada et al. 2004)—gene is visible in the 8-cell stage, where cytoskeletal dynamics and blastomere arrangement start to deviate from symmetry and do so in opposite directions in offspring from mothers carrying dextral versus sinistral genotypes. There is, however, a delay in asymmetry onset in sinistrals compared to dextrals (Shibazaki et al. 2004). Recently, it was found that such genetically determined gastropod chirality (including the downstream *nodal* expression patterns) can be reversed phenotypically by experimentally rotating embryonic cells in the opposite direction (Kuroda et al. 2009).

Because mating behaviour and reproductive anatomy in terrestrial gastropods are highly asymmetric, opposite dextral and sinistral morphs usually are prevented from interchiral mating (Gittenberger 1988), leading to chiral monomorphism (directional asymmetry) in this group (Schilthuizen and Davison 2005; Ueshima and Asami 2003). One exception to this rule was recently highlighted: almost all of the over 35 species of the subgenus *Amphidromus* Albers, 1850 are chirally dimorphic, with dextrals and sinistrals mixed in similar proportions in a population (Fig. 1). It was discovered (Schilthuizen et al. 2007) that this genetic anti-symmetry or chiral dimorphism could be maintained by sexual selection: improved sperm transfer in interchiral copulation (due to a better match between a dextral spermatophore and a sinistral oviduct or vice versa; Fig. 2b–d) would have selected for a preference for mating with partners of opposite coiling direction. Until now, however, it has not been clear whether the sperm cells themselves [which are, in Pulmonata, strongly asymmetrically shaped (Healy 1988); see further in Results and



Fig. 1 *Amphidromus inversus*. Sinistral (left) and dextral (right) shell

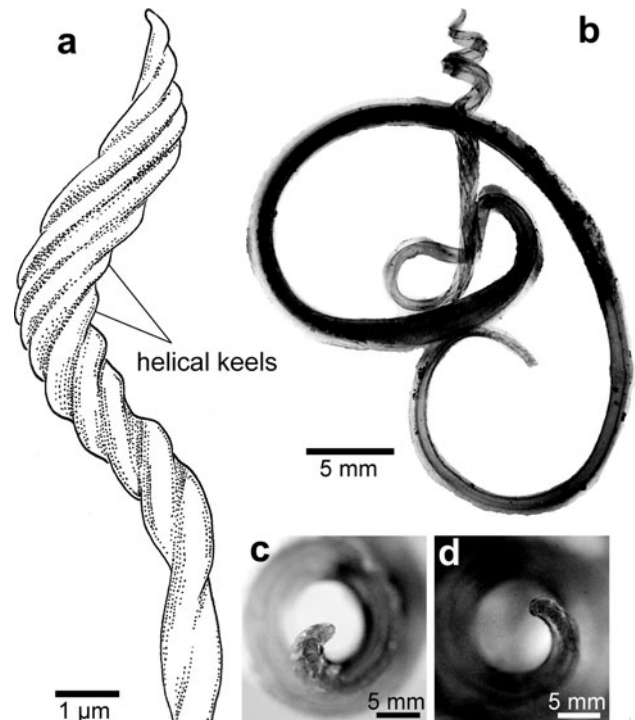


Fig. 2 *Amphidromus inversus*, spermatozoon and spermatophore. **a** Nucleus and anterior part of the midpiece (drawn after a scanning electron micrograph), showing the dextral helical elements (all studied spermatozoa, both from dextral-bodied and sinistral-bodied animals, have dextral chirality); **b** spermatophore with sinistraly-coiled ‘corkscrew’ tip, produced by a sinistral individual; **c–d** tip of a sinistral (**c**) and dextral (**d**) spermatophore, in apical view

Discussion] are chirally dimorphic as well. This is, however, not impossible; although studies in chirally dimorphic *Radix labiata* (Rossmässler, 1835) failed to find more than one chiral morph (Selman and Waddington 1953), other studies of pulmonate sperm morphology have found that both dextral and sinistral sperm morphs exist within a species, though it remains unclear whether correlations with shell coiling direction exist (see ‘Discussion’ for more details on this).

In this article, we study the morphology of sperm cells in dextral and sinistral individuals of *Amphidromus inversus* (Müller, 1774), for two reasons. First, the fact that maternally inherited cytoskeletal dynamics are mirror imaged in the blastomeres in gastropods suggests that a chiral molecule in the egg (the hypothesized ‘F molecule’ of Brown and Wolpert 1990) triggers chiral development. Hence, this chiral dimorphism may exist in sperm cells as well (and consequently aid in the discovery of the gene responsible). Second, swimming and turning behaviour of sperm cells may be influenced by the sperm morphology and the chirality of the travelling waves in the flagellum (Ishijima and Hamaguchi 1993; Ishijima et al. 1999), and it is conceivable that dextral sperm cells will be better able to navigate a

sinistral oviduct and vice versa, thus contributing to increased interchiral fertilization success. To avoid confusion between the chirality of the animal and its spermatozoa, we will indicate the whole-body chirality with ‘dextral-bodied’ or ‘sinistral-bodied’ throughout this article.

Materials and methods

As described earlier (Schilthuisen et al. 2007; Schilthuisen and Looijestijn 2009), we obtained entire spermatophores from copulating *Amphidromus inversus* (Gastropoda Pulmonata: Camaenidae) in the field on the island of Kapas, Malaysia. These are simultaneous hermaphrodites and produce, during a lengthy copulation, a single, large spermatophore in their male genitalia, which is then transferred to the partner’s female genitalia. This takes place reciprocally. We fixed copulating pairs in the field and maintained them in 70% ethanol until further study. Altogether, we had access to spermatophores produced by 6 dextral-bodied and 9 sinistral-bodied individuals. In some cases (where copulation had been interrupted early), the spermatophore was taken from the male genitalia of the sperm donor. In other cases, later in the copulation, the spermatophore was retrieved from the female genitalia of the sperm receiver. Since the spermatophore is coiled in the same direction as the animal that produces it (Schilthuisen et al. 2007), we could always confirm that a spermatophore in the female genitalia had the same chirality as the presumed sperm donor. We then used a scalpel to open each spermatophore and produce several fragments for which the spermatozoa containing content was exposed. These fragments were then dehydrated with acetone (2 × 30 min), dried in a critical-point dryer (BALZERS CPD 030), sputter-coated with gold in a BAL-TEC SCD005 and observed in a JEOL JSM-5300 scanning electron microscope (the numbers written on the specimen holder confirmed that the image in the microscope is not inverted). The chirality of each spermatozoon encountered was recorded.

Results

In all specimens, we found multiple spermatozoa. In all observed spermatozoa, the nucleus (head) carried four or five helically arranged keels starting directly posterior of the acrosome. The nucleus was slender, circa nine times as long as wide: mean width 1.33 μm (SD 0.15 μm ; min. 1.04 μm , max. 1.66 μm ; $N = 20$), mean length 11.7 μm (SD 1.17 μm ; min. 10.8 μm , max. 14.6 μm ; $N = 9$) and itself also spiral, consisting of approximately one loose helical whorl. In the anterior part of the midpiece, a single, coiled glycogen-filled helix compartment was visible,

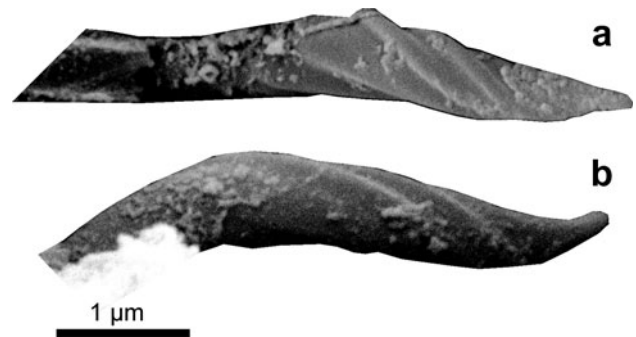


Fig. 3 *Amphidromus inversus*, scanning electron micrographs of nuclei of spermatozoa from a dextral-bodied (a) and a sinistral-bodied (b) individual. Both nuclei show a dextral coil

which was lacking from the much longer posterior part (mean diameter: 658 nm; SD 59 nm; min. 523 nm; max. 733 nm; $N = 20$) of the midpiece. Since only fragments of the spermatozoa were exposed, we could not determine the full length for entire spermatozoa. For each individual, we examined between 5 and 60 spermatozoa (total spermatozoa examined: 207) and found that the chirality of the nucleus, its helical keels and the glycogen helix was clearly dextral in all cases (Figs. 2a, 3).

Discussion

The shape and dimensions of the *Amphidromus inversus* spermatozoon, with its slender, screw-like head and long glycogen helices along the midpiece is similar to the complex, asymmetric spermatozoa reported for other Pulmonata (e.g. Thompson 1973). These spiral arrangements develop during the late stage of spermiogenesis (Watts 1952), initially in the nucleus, then in the midpiece (Hickman 1931), a process apparently aided by a twisted sheath of microtubuli that lies around the mitochondrial derivative (Healy 2001). Unfortunately, only rarely in the rich literature on pulmonate sperm morphology is the coiling direction of the helical elements explicitly mentioned [not even by Thompson (1973), who dwells at length on the functional significance of coiled gastropod spermatozoa], and chirality cannot be determined unambiguously from published transmission electron micrographs, only from scanning electron micrographs. Yet, what is available in the literature is sufficient to state that the chiral uniformity found in *A. inversus* is not representative for Pulmonata as a whole.

In some species, like in *A. inversus*, coiling direction of the helical elements of the spermatozoa appears to be consistently dextral, as is the case in the dextral-bodied *Arion hortensis* Férussac, 1819, *Agriolimax reticulatus* (Müller, 1774), *Milax sowerbyi* (Férussac, 1823), *Siphonaria*

japonica (Donovan, 1834), *S. sirius* (Pilsbry, 1894), and *Anguispira alternata* (Say, 1816) (Anderson 1982; Maxwell 1975; Bayne 1970; Sumikawa and Funakoshi 1984) and in the sinistral-bodied *Bulinus globosus* (Morelet, 1866), *B. africanus* (Krauss, 1848), and *Physella acuta* (Draparnaud, 1805) (Appleton and Brackenbury 1997; Brackenbury and Appleton 1991). In other species, the coiling is sinistral, like in the (dextral-bodied) terrestrial species *Cornu aspersum* (Müller, 1774), *Oxyloma elegans* (Risso, 1826) and *Testacella* Draparnaud, 1801 sp. (Anderson and Personne 1967; Maxwell 1975; Watts 1952; Selmi et al. 1989). In other species, spermatozoon chirality appears to be mixed: whereas Selman and Waddington (1953) describe the nuclear helical ridges and glycogen helices of 12 sperm cells from sinistral-bodied *Radix labiata* (Rossmässler, 1835) and eight sperm cells from dextral-bodied *R. labiata* to be all dextral, the scanning electron micrographs of *R. labiata* spermatozoa in Maxwell (1975) show sinistral coils on both the nucleus and the midpiece [as do the splendid freeze-etch electron micrographs of Thompson (1973) for the dextral-bodied *L. stagnalis*]. Hickman (1931: 265) states that in the dextral-bodied *Novisuccinea ovalis* (Say, 1817), ‘there is no uniformity of direction of the spirals. [...] Out of many specimens examined, I find that the spirals may go in either a left or a right direction from head to tail, and that one condition is about as common as the other’ and a similar observation is made for the dextral-bodied *Arion subfuscus* (Draparnaud, 1805) by Watts (1952).

The above—admittedly very limited—review of the literature and our new data for *A. inversus* show clearly that spermatozoon chirality may be fixed within species, or dimorphic, even within an individual, and that it is not correlated with whole-body chirality in any obvious way. This means that it is unlikely that the cell ultrastructure that gives rise to the helical twists in the developing spermatid has the same origin as the one that sets off chiral development in the embryo. Similarly, it is unlikely that spermatozoon chirality plays a role in the maintenance of whole-body chiral dimorphism in *Amphidromus inversus*.

Yet, our data do generate new and interesting questions about the evolution and genetics of spermatozoon chirality. First, the fact that chiral dimorphism exists in some species and chiral monomorphism in others suggests that the chirality of components of the cell ultrastructure in early spermatids evolves rapidly and can shift easily between heritable and random orientation. Second, as Thompson (1973), using scale models of gastropod spermatozoa in glycerol, has shown that the helical shape of the nucleus and midpiece provide a means of converting uniplanar flagellar activity into helical forward movement, then swimming and turning behaviour of the active spermatozoa may be influenced by the chirality. In

addition, in human sperm, spontaneous alternation behaviour has been recorded, which makes sperm cells, when forced to turn in a maze, preferentially turn in the opposite direction at the first opportunity (Brugger et al. 2002). It is thus not inconceivable that sperm chirality will have an effect on the escape rate of the spermatozoa from the gametolytic organ, on sperm competition and on oocyte penetration.

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