MALAKOLOGISCHE ABHANDLUNGEN
Staatliches Museum für Tierkunde Dresden

Band 20 | Ausgegeben: 25. März 2002 | Nr. 25

A sinistral specimen of the terrestrial slug *Arion lusitanicus* (Gastropoda: Pulmonata: Arionidae)

With 2 figures

HEIKE REISE, MANDY BENKE & JOHN M. C. HUTCHINSON

Abstract. A juvenile sinistral specimen of *Arion lusitanicus* was found near Münster (Germany, Nordrhein-Westfalen) and raised in isolation. Its entire external and internal morphology is a mirror image of normal dextral slugs. In mating experiments with dextral individuals the partners showed clear courtship behaviour but were never successful. This was probably caused by the opposite position of their genital pores, which might make copulation impossible for mirror-image slugs. Reports of sinistral terrestrial slugs are very rare. The authors review earlier cases, and discuss the possible genetic basis of sinistrality in slugs.

Kurzfassung. **Ein sinistrales Individuum der terrestrischen Nacktschnecke *Arion lusitanicus* (Gastropoda: Pulmonata: Arionidae).** – Ein sinistrales Jungtier von *Arion lusitanicus* wurde bei Münster (BRD, Nordrhein-Westfalen) gesammelt und isoliert aufgezogen. Die gesamte innere und äußere Morphologie des Tieres ist spiegelbildlich zur normalen, dextralen Morphe. Bei Verpaarungsversuchen mit dextralen Individuen zeigten die Tiere klares Paarungsverhalten, waren aber immer erfolglos. Die Ursache dafür ist wahrscheinlich die unterschiedliche Position der Genitalöffnungen, die spiegelseitigen Partnern bei Nacktschnecken grundsätzlich eine Verpaarung unmöglich machen könnte. Berichte über sinistrale terrestrische Nacktschnecken sind sehr selten. Die Autoren geben eine Übersicht über frühere Berichte und diskutieren mögliche genetische Grundlagen von Sinistralität bei terrestrischen Nacktschnecken.

Key words. Arionidae, *Arion lusitanicus*, sinistrality, handedness, reproductive isolation, mating, dextral.

The handedness of shell coiling in snails has attracted the attention of natural historians for a long time, and many publications deal with this phenomenon (e.g. BOYCOTT et al., 1930; GOULD & YOUNG, 1985; GALLOWAY, 1987; ROBERTSON, 1993; ASAMI et al., 1998). Owing to the asymmetrical bauplan of the body, snail shells are either right-handed (dextral) or left-handed (sinistral). The large majority of gastropod species are dextral, having their pneumostome, genital and excretory pores on the right (CLARKE et al., 1978; ASAMI, 1993; ROBERTSON, 1993). Some groups, such as the Clausiliidae, consist mainly of sinistral species. But very few species are known to be polymorphic in coiling, that is with both sinistral

Authors' addresses:
Dr. Heike Reise, Staatliches Museum für Naturkunde Görlitz,
PF 300 154, D-02806 Görlitz; e-mail: smng.reise@t-online.de
Mandy Benke, Ossenkampstiege 97, D-48163 Münster
Dr. John M.C. Hutchinson, Max-Planck-Institut für Bildungsforschung,
Lentzeallee 94, D-14195 Berlin

Fig. 1: Adult sinistral specimen (lighter) together with a normal *A. lusitanicus* from the neighbourhood of Görlitz (Germany, Saxony).

and dextral shells commonly occurring (ASAMI, 1993). However, in many snail species exceptional cases of shells with the opposite orientation occur (e.g. ANCEY, 1906; SEIDL, 1989; ROBERTSON, 1993; PEREZ & ESPINOSA, 1994).

The handedness is less obvious in slugs since they lack the typical snail shell, and their visceral mass is incorporated into the head-foot. However, the internal arrangement of the organs and the position of the body openings on the right show that all terrestrial slugs are dextral. Surprisingly, and in contrast to snails, reports about sinistral specimens in slugs are very rare, raising the question whether sinistrals really occur less often in slugs, or whether they are merely more readily overlooked. The only reports known to us concern single sinistral specimens of *Arion rufus* (L., 1758) (BAUDON, 1884), *Arion intermedius* Normand, 1852 (DAVIES, 1972), *Milax gagates* (Draparnaud, 1801) (DAVIES, 1973) and *Bielzia coerulans* (Bielz, 1851) (SEIBERT, 1873). A few papers deal with unusual body deformations, for instance in *Arion* (SIMROTH, 1905; BOETTGER, 1956), *Urocyclus* (SIMROTH, 1905) and *Limax* (WIKTOROWA, 1962), but these have nothing to do with handedness. Sinistrality is completely unknown in marine slugs (ROBERTSON, 1993). In this paper, we report a sinistral *Arion lusitanicus* Mabille, 1868.

On 17th April 2000, MB collected a single juvenile slug from grassland along the river Werse near Werse, NE of Münster (Germany, Nordrhein-Westfalen). This slug was taken to the State Museum of Natural History Görlitz, and, because its pneumostome was on the left (Fig. 1), was raised there in isolation in a temperature chamber (15°C, LD 12 : 12). The juvenile slug was brown with greyish sides and dark lateral stripes, and changed later into an orange adult lacking stripes.

Between 10th October and 1st November we set up mating trials between the sinistral slug and two normal (i.e. dextral) *A. lusitanicus* and one dextral *A. rufus* (species identities were determined later by dissection) collected as adults in the neighbourhood of Görlitz (Germany, Saxony). After several days of isolation to increase their readiness to mate, each dextral slug was put together with the sinistral specimen, and kept under observation (direct or with a video camera) for 2 to 23 h. In the two pairings with *A. rufus* neither of the partners showed any interest. However, with each *A. lusitanicus* partner we observed typical patterns of early courtship behaviour in one out of four and two out of two experiments. In such

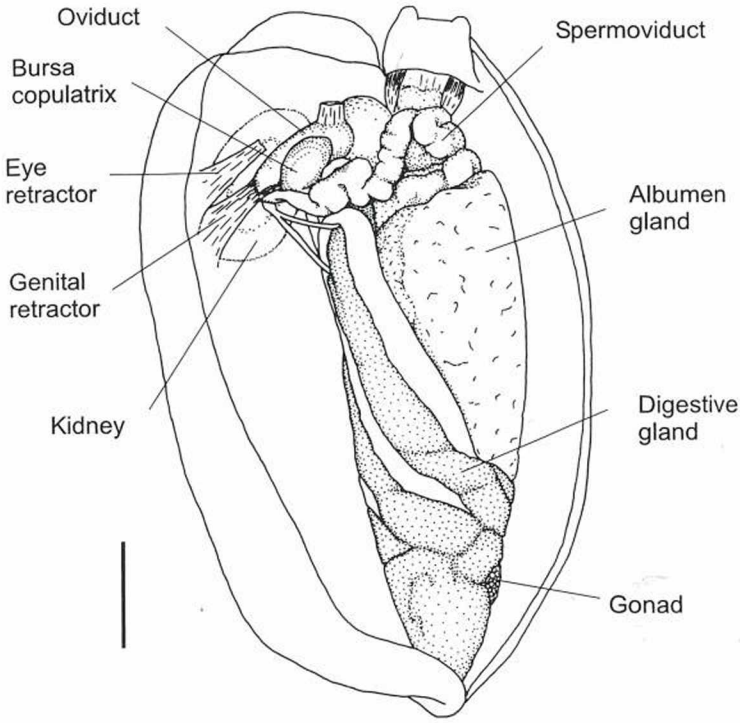

Fig. 2: Internal morphology of the sinistral specimen. Scale bar = 10 mm.

cases the couple showed repeated courtship behaviour, lasting up to 45 min and including trail following, circling, and licking of the partner's tail gland, genital pore and face. Usually the genital pores were swollen, and sometimes showed a white mass (the beginning of the eversion of the atrium). However, the partners showed clear signs of confusion over positioning relative to each other, and mating never proceeded further. The dextral slugs tended to circle clockwise while the sinistral slug almost always circled counterclockwise. One of the dextral *A. lusitanicus* partners was later allowed to mate with another dextral conspecific. These partners quickly began circling in a clockwise direction, and then assumed the usual Ying-Yang position, although in the end they did not copulate. However, this shows at least that positioning was no problem with the right partner.

The sinistral slug was killed in 50% ethanol at the beginning of November, and dissected for species determination, as well as to investigate its internal organisation. The specimen is ca. 63 mm long (in ethanol), and the albumen gland is very large, although the slug never laid eggs. An anatomical investigation revealed the internal organs to be a complete mirror image of the usual arrangement in *A. lusitanicus*, including the position of the genitalia, the loops of the digestive tract, the rectal opening, and the pallial complex (Fig. 2). The specimen is stored in the collection of the State Museum of Natural History Görlitz (catalogue number p9075).

Other than in trauma-induced shell-coiling inversions with a normal organisation of the soft body (e.g. HARASEWYCH, 1998), the coiling direction of gastropods is determined by the direction of spiral cleavage, and can be apparent even at the first cleavage (FREEMAN &

LUNDELIUS, 1982; ROBERTSON, 1993). At least in some species, variation in coiling direction is genetically determined. The few cases studied so far have revealed alternative alleles at a single locus, dominance of the common allele, and delayed expression in which the phenotype depends on the mother's genotype (BOYCOTT et al., 1930; MURRAY & CLARKE, 1966; FREEMAN & LUNDELIUS, 1982; ASAMI, 1993; and references therein). Furthermore, FREEMAN & LUNDELIUS (1982) demonstrated that sinistrality in *Lymnaea peregra* is caused by the absence of a maternal gene product in the egg cytoplasm that normally induces dextrality. If one assumes the same genetic background for *A. lusitanicus*, one might expect more sinistral individuals to turn up in the Münster population. However, a search at the same locality in autumn 2001 was unsuccessful, although normal dextral slugs of this species were very common.

At least two of the four papers on sinistral slugs seem to suggest a different genetic background to their sinistrality. The sinistral individuals of *M. gagates* and *B. coerulans* were each F1-offspring of a dextral wild individual, and they hatched together with respectively two and an unreported number of dextral siblings (SEIBERT, 1873; DAVIES, 1973). Similarly, the sinistral specimen of *A. intermedius* was an F2-offspring of a dextral wild specimen hatching together with an unreported number of dextral individuals (DAVIES, 1972), although it is possible that the latter were not siblings of the sinistral specimen. At least in the first two cases, delayed expression of a sinistral maternal genotype would seem to be excluded, as all siblings should then have been sinistral. However, FREEMAN & LUNDELIUS (1982) explained similar isolated occurrences of sinistral *Lymnaea peregra* in dextral families as due to incomplete penetrance when the mother was heterozygote (the dosage of the dextral-inducing substance was then low and in some eggs might fall below the critical threshold). Other potential explanations are delayed expression of a mutation in one cell line during oogenesis in a heterozygote mother, direct expression of a mutant embryo genotype, or a non-genetic phenomenon. The latter two explanations are known in other taxa (e.g. direct expression in vertebrates, and alterations of handedness by temperature, removal of the egg shell, or mechanical manipulations in nematodes – WOOD et al., 1996) but we know of no evidence in molluscs or other phyla with spiral cleavage.

Unfortunately, we were not able to investigate the genetics of sinistrality in *A. lusitanicus* as our specimen failed to mate with dextral conspecifics and thus to reproduce. Self-fertilization seems not to be known in *A. lusitanicus* (GRIMM, 2001, pers. comm.). Generally in internally-fertilizing gastropods the exact positioning of genital pores next to one another in normal pairings demands a sequence of elaborate co-ordinated behaviours that do not work properly when the individuals are mirror images. The consequent reproductive isolation is incomplete in snails with high-spired shells, while low-spired snails may be unable to mate with each other at all because in these species the body orientations are normally antiparallel (GITTENBERGER, 1988; ASAMI et al., 1998). Inability to mate with the opposite coiling morph causes strong selection against the rare morph (JOHNSON, 1982; ASAMI et al., 1998). However, delayed expression and recessiveness may allow the rare coiling allele to persist in the population for a long time (JOHNSON et al., 1990; VAN BATENBURG & GITTENBERGER, 1996) and occasionally even become fixed (VAN BATENBURG & GITTENBERGER, 1996). The question of whether coiling polymorphism might be important for sympatric speciation has been discussed (GITTENBERGER, 1988; JOHNSON et al., 1990; ANISTRATENKO & BAIDASHNIKOV, 1991; VAN BATENBURG & GITTENBERGER, 1996; ASAMI et al., 1998). Gastropods can show a reduced propensity for mating with conspecifics from different populations (e.g. BAUR & BAUR, 1992), so we cannot exclude that the failure of the mating attempts between our sinistral and dextral slugs is due to their different origins. However, the behaviour implied rather that the incompatibility was caused by the opposite position of their genital pores. Slugs of the genus *Arion* mate on the ground, assuming a Ying-Yang position so that the partners' genital pores face each other. Genitalia are everted without intromission, and a successful

(usually reciprocal) spermatophore transfer probably depends much on the exact positioning of their genital pores relative to each other. For this reason, we suggest that mating between slugs with antiparallel orientation is as impossible as in snails with low-spired shells. (The only exception might be species that can mate in a parallel orientation, such as some limacids that mate while hanging tightly entwined on a vertical mucus string.) Such incompatibility might be a reason for the rarity of sinistral slugs, if we assume that sinistrality is genetically based. On the other hand, many slugs can self-fertilize, which would allow a single sinistral individual to reproduce. It thus remains highly desirable to perform breeding experiments with a self-fertilizing slug species, although the precondition would be the discovery of a sinistral specimen of such a species.

Acknowledgements

Many thanks to T. Backeljau for helpful comments on the manuscript and for highly valuable help with literature. J. Wojan helped with the mating experiments. We are also grateful to A. Norris who drew our attention to the cited publications by Davies.

References

ANCEY, C.-F. (1906): Observations sur les mollusques gastéropodes sénestres de l'époque actuelle. – Bull. Scient. Fr. Belg. **40**: 187–205.

ANISTRATENKO, V.V. & BAIDASHNIKOV, A.A. (1991): Evolutionary significance of the coiling inversion in mollusks. – Vestn. Zool. **2**: 10–14 [in Russian].

ASAMI, T. (1993): Genetic variation and evolution of coiling chirality in snails. – Forma **8**: 263–276.

ASAMI, T., COWIE, R.H. & OHBAYASHI, K. (1998): Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. – Am. Nat. **152**: 225–236.

BAUDON, A. (1884): Troisième catalogue des Mollusques vivants du département de l'Oise. – J. Conch., Paris **32**: 193–325.

BAUR, B. & BAUR, A. (1992): Reduced reproductive compatibility in *Arianta arbustorum* (Gastropoda) from distant populations. – Heredity **69**: 65–72.

BOETTGER, C.R. (1956): Über einen Fall von pathologischer Gestaltsveränderung bei einer Wegschnecke der Art *Arion ater* (L.). – Biol. Zbl. **75**: 257–267.

BOYCOTT, A.E., DIVER, C., GARSTANG, S.L. & TURNER, F.M. (1930): The inheritance of sinistrality in *Limnaea peregra* (Mollusca, Pulmonata). – Phil. Trans. R. Soc. London B **219**: 51–131.

CLARKE, B., ARTHUR, W., HORSLEY, D.T. & PARKIN, D.T. (1978): Genetic variation and natural selection in pulmonate molluscs. In: FRETTER, V. & PEAKE, J. (eds.): Pulmonates, Vol. 2A. Systematics, evolution and ecology: 219–270. London: Academic Press.

DAVIES, S.M. (1972): A sinistral *Arion intermedius* Normand. – Conch. Newsl. **40**: 251.

DAVIES, S.M. (1973): Another sinistral slug – *Milax gagates* (Draparnaud). – Conch. Newsl. **45**: 318.

FREEMAN, G. & LUNDELIUS, J.W. (1982): The developmental genetics of dextrality and sinistrality in the gastropod *Lymnaea peregra*. – Roux Arch. Devel. Biol. **191**: 69–83.

GALLOWAY, J. (1987): A cause for reflection? – Nature **330**: 204–205.

GITTENBERGER, E. (1988): Sympatric speciation in snails: a largely neglected model. – Evolution **42**: 826–828.

GOULD, S.J., YOUNG, N.D. & KASSON, B. (1985): The consequences of being different: sinistral coiling in *Cerion*. – Evolution **39**: 1364–1379.

GRIMM, B. (2001): Biology of the pest slug *Arion lusitanicus* in central Europe. In: SALVINI-PLAWEN, L., VOLTZOW, J., SATTMANN, H. & STEINER, G. (eds.): Abstracts, World Congress of Malacology 2001: 133. Vienna: Unitas Malacologica.

HARASEWYCH, M.G. (1998): Trauma-induced, *in utero* hyperstrophy in *Melanoides tuberculata* (Müller, 1774). – J. Moll. Stud. **64**: 404–405.

JOHNSON, M.S. (1982): Polymorphism for direction of coil in *Partula suturalis*: behavioural isolation and positive frequency dependent selection. – Heredity **49**: 145-151.

JOHNSON, M.S., CLARKE, B. & MURRAY, J. (1990): The coil polymorphism in *Partula suturalis* does not favor sympatric speciation. – Evolution **44**: 459–464.
MURRAY, J. & CLARKE, B. (1966): The inheritance of polymorphic shell characters in *Partula* (Gastropoda). – Genetics **54**: 1261–1277.
PEREZ, A.M. & ESPINOSA, J. (1994): Sinistralidad en *Caracolus sagemon marginelloides* (Orbigny in Sagra, 1847) (Mollusca: Gastropoda: Camaenidae). – Cuad. Invest. Biol. (Bilbao) **18**: 235–244.
ROBERTSON, R. (1993): Snail handedness. – Natl. Geogr. Res. Expl. **9**: 104–119.
SEIBERT, H. (1873): Zur Kenntnis unserer Nacktschnecken. – Malakozool. Bl. **21**: 190–203.
SEIDL, F. (1989): Sinistrale und dextrale Anomalien bei mitteleuropäischen Gehäuseschnecken (Gastropoda, Pulmonata). – Malak. Abh. Mus. Tierkde. Dresden **14**: 103–104.
SIMROTH, H. (1905): Über zwei seltene Mißbildungen an Nacktschnecken. – Z. wiss. Zool. **82**: 494–522.
VAN BATENBURG, F.H.D. & GITTENBERGER, E. (1996): Ease of fixation of a change in coiling: computer experiments on chirality in snails. – Heredity **76**: 278–286.
WIKTOROWA, J. (1962): An interesting anomaly in *Limax cinereo-niger* Wolf. (Limacidae, Pulmonata). – Zool. Polon. **12**: 101–106, plates I, II.
WOOD, W.B., BERGMANN, D. & FLORANCE, A. (1996): Maternal effect of low temperature on handedness determination in *C. elegans* embryos. – Develop. Genetics **19**: 222–230.

(Received on December 4, 2001)