

Mapping of dextral : sinistral proportions in the chirally dimorphic land snail *Amphidromus inversus*

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One of the very few snail taxa that display genetic antisymmetry (that is, roughly equal mixes of genetically determined clockwise [D] and anticlockwise [S] coiled individuals within a single population) are the circa 35 species of the tropical tree snail subgenus *Amphidromus*. Previous work has shown that this may be due to a particular type of sexual selection, in which sperm transfer is improved in copulations between the two mirror-image morphs. However, it is not yet clear why so often significant deviations from 50:50 proportions are found. Modelling studies show that population structure will affect the degree by which the dimorphism is skewed towards the morph associated with the recessive allele. In this study, we mapped the proportions of sinistrals (PropS) in 56 demes in *A. inversus* on the Malaysian island of Kapas. We also mapped population density, predation rates, and several measures of vegetation structure. Our results show that PropS amounts on average to 0.65, but across the island it varies from 0.30 to 0.85. Density and overall predation are inversely correlated. A general linear model selection procedure results in the proportion of sinistrals to be positively correlated with density and predation on dextrals. We find no overwhelming evidence for a role for drift in explaining the deviations from equal S:D proportions, but we do argue that further study of crab-snail interactions may be warranted.

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3 ***inversus***

4 Menno Schilthuizen,^{1,2*} Sylvia Looijestijn,³ Sek Chuan Chua,⁴ Jesús Aguirre Gutiérrez,^{1,5} and Ruth F.
5 Castillo Cajas⁶

6 ¹ Naturalis Biodiversity Center, Character Evolution Group, Darwinweg 2, 2333 CR Leiden, the
7 Netherlands

8 ² Institute Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE Leiden, the Netherlands

9 ³ Independent field biologist, Oranjelaan 35, 2635 JK Den Hoorn, the Netherlands

10 ⁴ Independent field biologist, P.O. Box 300, Bukit Batok Central, 916510 Singapore, Republic of
11 Singapore

12 ⁵ Institute for Biodiversity and Ecosystems Dynamics, Computational Geo-Ecology, University of
13 Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands

14 ⁶ Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, D-
15 97074 Würzburg, Germany

16 *Corresponding author: +31-6-22030313; menno.schilthuizen@naturalis.nl

17

18 **ABSTRACT**

19 One of the very few snail taxa that display genetic antisymmetry (that is, roughly equal mixes of
20 genetically determined clockwise [D] and anticlockwise [S] coiled individuals within a single
21 population) are the circa 35 species of the tropical tree snail subgenus *Amphidromus*. Previous work
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25 structure will affect the degree by which the dimorphism is skewed towards the morph associated with
26 the recessive allele. In this study, we mapped the proportions of sinistrals (PropS) in 56 demes in *A.*
27 *inversus* on the Malaysian island of Kapas. We also mapped population density, predation rates, and

several measures of vegetation structure. Our results show that PropS amounts on average to 0.65, but across the island it varies from 0.30 to 0.85. Density and overall predation are inversely correlated. A general linear model selection procedure results in the proportion of sinistrals to be positively correlated with density and predation on dextrals. We find no overwhelming evidence for a role for drift in explaining the deviations from equal S:D proportions, but we do argue that further study of crab-snail interactions may be warranted.

INTRODUCTION

The helically coiled body (and shell) of snails (Gastropoda) is one of their chief distinguishing features, making them one of the few bilaterian animal groups with whole-body asymmetry (Schilthuizen & Davison, 2005). Like all three-dimensional, bilaterally asymmetric forms, the body of a snail may thus come in two forms (enantiomorphs): dextrally (clockwise; D) coiled, or sinistrally (anticlockwise; S) coiled. The majority of gastropod species is composed entirely of dextral individuals, a minority of species are sinistral, and only in a minute proportion of species do both enantiomorphs exist side by side (Vermeij, 1975; Gittenberger, Hamann & Asami, 2012). The best example for this latter category comes from the Southeast-Asian genus *Amphidromus* (Camaenidae) (Asami, Cowie & Ohbayashi, 1998). This genus of large, often colourful tree snails consists of several subgenera (Sutcharit & Panha, 2006; Laidlaw & Solem, 1961), one of which, *Amphidromus* s. str., comprises ca. 35 species that are almost all chirally dimorphic: dextral and sinistral individuals occur at appreciable frequencies within each species, and usually also within each population (Sutcharit, Asami & Panha, 2007; Schilthuizen et al., 2007).

Since in most land snail species, copulation between mirror-imaged individuals is impeded (Gittenberger, 1988; Asami, Cowie & Ohbayashi, 1998), chiral dimorphism is expected to be an evolutionarily unstable situation (Van Batenburg & Gittenberger, 1996). Therefore, over the past decade, the chiral dimorphism in *Amphidromus* has received much attention (Schilthuizen et al., 2007; Sutcharit, Asami & Panha, 2007; Craze, Elahan & Schilthuizen, 2006; Nakadera et al., 2010), especially in the species *A. inversus* (Fig. 1). A series of studies has shown that, like in other pulmonate snails (Schilthuizen & Davison, 2005; Boycott et al., 1931; Murray & Clarke, 1966), the coiling direction is determined by delayed, single-gene inheritance and full dominance (Schilthuizen et al.,

2007; Schilthuisen & Looijestijn, 2009). Chiral dimorphism is present at all spatial scales (Schilthuisen et al., 2007), and S:D mixes are found even in individual trees (Schilthuisen & Looijestijn, 2009). No indication of niche differentiation can be observed (Schilthuisen et al., 2012), and dispersal is equal between both morphs and sufficient to assure the mixing of populations at a spatial scale larger than that of individual trees (Schilthuisen et al., 2005, 2012). These observations suggest that balancing selection must be maintaining the dimorphism. However, preferences for the commoner morph by rodent predators, which could have provided such balancing selection, were not found (Schilthuisen et al., 2007).

A possible solution for this evolutionary conundrum was found in sexual selection. Unlike the situation in other land snails, interchiral (SxD) copulation in *Amphidromus* is not impeded (Schilthuisen & Looijestijn, 2009). In fact, a slight preference appears to exist for dextrals to mate with sinistrals and vice versa. Moreover, the morphologies of the spermatophore, the spermatophore-receiving organ, and the oviduct are such that it may be surmised that sperm transfer is improved in such interchiral copulation. Together, these two factors provided sufficient balancing selection for a simulation model to predict that dimorphism should be achieved (Schilthuisen et al., 2007).

However, three problems remain with this explanation for the maintenance of chiral dimorphism in *Amphidromus*. First, the observations in *A. inversus* need to be repeated in other species of *Amphidromus* s. str. Second, higher interchiral fertilization rate must be demonstrated, rather than inferred. And finally, the model predicts equal S:D proportions, whereas in natural populations, deviations from 50:50 proportions are usually found. In this paper, we deal with the latter problem.

In chirally dimorphic *Amphidromus* species, a broad range of relative S:D proportions has been reported. Sutcharit, Asami & Panha (2007) found significant deviations (in both directions) from equal proportions in populations of at least four *Amphidromus* taxa from Thailand. They also report shifts in proportions in the same population over periods of up to ten years. Similarly, Craze, Elahan & Schilthuisen (2006) found a deviation in one *A. martensi* population in Borneo. On the island of Kapas, the field locality for much of the previous work on *A. inversus*, proportions of sinistrals (PropS) at two separate sites appear to be stable at 65 % sinistrals (Schilthuisen et al., 2007).

Craze (2009) suggested that, under a fragmented population structure, deviations from balanced dimorphism may be expected due to drift. He produced a simulation model, which showed that, while

balancing selection would produce equal S:D proportions in a panmictic population of infinite size, this is not the case in smaller, fragmented, natural populations. In such cases, the recessive allele (which, to produce phenotypic 50:50 conditions, must have a higher frequency in the population than the dominant allele) will be fixed by drift more often than the dominant (and therefore rarer) allele.

In this paper, we explore the possibility that drift may explain the deviation from equal S:D proportions in *A. inversus*. We map PropS throughout the island of Kapas, and we also map population density, predation rate, and several vegetation characteristics. Regardless of the fact that it is not yet clear whether the sinistral or the dextral allele is recessive in *A. inversus*, our prediction would be that any deviations from equal proportions would be particularly strong under environmental conditions that reduce interdemetic dispersal.

MATERIALS AND METHODS

Between October 28th and November 3rd, 2013, we visited 56 study sites throughout the (2.3 x 1.0 km) island of Kapas, off the coast of Terengganu, Malaysia. At each study site, we searched a circular area with a 25 m radius for 22.5 or 45 person minutes. Search was carried out by one, two, or three persons, all experienced field ecologists. We devoted equal amounts of time to searching the forest floor and the vegetation. Both empty shells and live snails were taken, of all age categories. After the search was completed, we recorded the numbers of each coiling morph, and differentiated between live and dead snails, and, within the latter group, between damaged and intact specimens. Damaged shells always showed characteristic predation damage by either rodents (top whorls broken off) or crustaceans (whorl walls peeled off), so all damaged specimens were considered to have died due to predation. After recording the data, all snails (dead and alive) were returned to the study site. Finally, we recorded the following data for each study site: latitude and longitude (by hand-held GPS), estimated maximum canopy height, density of climbers and vines (four categories from 1, sparse, to 4, dense), and percentage of understory ground cover.

We first performed exploratory linear regressions among S:D proportions, density, predation rate, relative predation on sinistrals and vegetation characteristics. We then mapped density, predation rate, and PropS as follows. To correct for the fact that our sample number was relatively small ($n = 56$), and

that low densities might have a disproportionately large effect on the calculated rates, we included a weighting procedure which consisted of repeating the variable of interest (e.g., predation rate), n times and randomly placing these values within a 25 m radius of where it originally occurred (x position), where n represents the total number of individuals sampled at x position. By doing this, we removed the influence of higher rates when the sample sizes are small (e.g., 1 individual out of 3). We used this “increased dataset” to plot the maps shown for predation and PropS (not for density). To conduct all plots we used R version 3.0.2 and the following packages: *sp* (Pebesma & Bivand, 2005; Bivand, Pebesma & Gómez-Rubio, 2008), *maptools* (Bivand et al., 2013), *rgdal* (Keitt et al., 2011), *gstat* (Pebesma, 2004), *rgeos* (Bivand & Rundel, 2013), *plotKML* (Hengl et al., 2013), and *plotrix* (Lemon, 2006).

To investigate how the environmental variables may correlate with the S:D proportions, we applied a generalized linear model (GLM) with binomial error structure using the number of sinistral over total snails as response variable and the environmental variables Density, predT (total predation rate), predD (predation rate on dextrals), predS (predation rate on sinistrals), canopy height, fvine (vine density), and gcnun (ground cover) as explanatory variables. We tested all two-way interactions between the explanatory variables. We then selected the most parsimonious model (smallest corrected Akaike Information Criterion, AICc) by applying a model selection procedure using the R package “MuMIn” (Barton, 2014) and by keeping all models with $\Delta < 2$. All statistical analyses were implemented in the R platform (R Development Core Team, 2014).

RESULTS AND DISCUSSION

We found snail densities that ranged from 0.0 to 5.6 snails per person per minute. Proportions of predation among the empty shells (counting only samples > 9 empty shells) ranged between 0.1 and 0.8. Proportions of sinistrals (PropS; only counting samples > 9) varied between 0.36 and 0.83, although in low-density areas these proportions will have been subject to relatively large sampling error. PropS for all samples pooled (914 sinistrals out of 1392 snails) amounted to 0.65, a proportion identical to those reported previously for this *A. inversus* population (Schilthuizen et al., 2007). Raw data are provided in Supplementary File 1.

Mapping of density (Fig. 2a), predation (Fig. 2b), and PropS (Fig. 2c) reveals considerable geographic variability. Density was high on parts of the west coast, and low elsewhere. Predation showed the reverse pattern: low on parts of the west coast, high elsewhere. Indeed, density and predation are significantly negatively correlated ($r = -0.65$; $P < 0.001$; Fig. 3). A simple explanation for this pattern might be that population densities increase when mortality is low thanks to reduced predator density. However, since the high-density/low-predation areas also coincide with low-lying areas with a soil composed mainly of calcium-rich coral sand, we may consider that the calcium content of the soil is what increases the snail densities (Graveland et al., 1994; Kappes & Topp, 2014) and that reduced predation due to predator satiation is then a consequence. However, these causative relations can only be proven with direct experiments which have yet to be performed. Overall, our mapping study shows that PropS appears to be more or less stable throughout the island around 0.65, although there is some geographic variation in this (which may be partly due to stochasticity due to small sample sizes).

Our GLM analysis (full model and details on model selection in Supplementary File 1) resulted in two optimal models. The most complete of these shows that PropS increases with higher PredD (coefficient = 0.57) and with higher density (coefficient = 0.21). Since no vegetation parameters show up in either model, this is contrary to the hypothesis that environmentally-driven population fragmentation drives deviation from 50:50 proportions (Craze, 2009). However, the vegetation characteristics that we measured were estimated by the researchers in the field, and a GIS study with, e.g., LiDAR data (Jung et al., 2012) might reveal patterns that have remained hidden in the present study.

Our finding of a positive correlation between PropS and PredD may warrant further study as well. It appears that in areas with increased predation of dextrals, proportions of sinistrals go up as a result. Our earlier studies (Schilthuizen et al., 2007) showed no such preferences for rat predation. However, in the present study predation comprised both rat and crab predation. This might indicate that crab predation may be non-random with respect to coiling morph. This would also fit with the observation from our GLM that PropS is positively correlated with density: crabs are chiefly present along the west-coast beach, where also density is the highest. These considerations, combined with the well-known crab claw chirality (Dietl & Hendricks, 2006), would suggest that further study into the crab-snail interaction may prove fruitful, and we are currently undertaking such studies.

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245

246 FIGURES

247 **Figure 1.** A sinistral (on left) and a dextral (on right) shell of *A. inversus* from Pulau Kapas (shell
248 height = ca. 40 mm).

249 **Figure 2.** Maps showing (a) the density distribution of *A. inversus* on Pulau Kapas (unit = snails per
250 person-minute search time); (b) weighted predation rates in *A. inversus* on Pulau Kapas (proportion of
251 empty shells with predation damage); (c) weighted distribution of proportion of sinistrals in *A. inversus*
252 on Pulau Kapas.

253 **Figure 3.** Graph showing population density in *A. inversus* on Pulau Kapas in relation to predation ($r =$
254 -0.65 ; $P < 0.001$)

1

Image of dextral and sinistral *Amphidromus inversus*

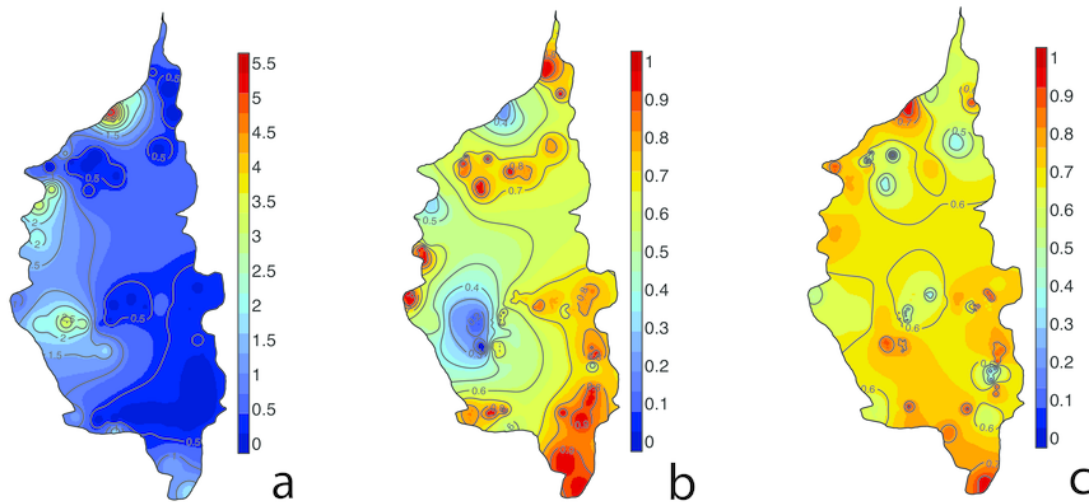
A sinistral (on left) and a dextral (on right) shell of *A. inversus* from Pulau Kapas (shell height = ca. 40 mm).



2

Maps of density, predation and sinistral proportions on Pulau Kapas

Maps showing (a) the density distribution of *A. inversus* on Pulau Kapas (unit = snails per person-minute search time); (b) weighted predation rates in *A. inversus* on Pulau Kapas (proportion of empty shells with predation damage); (c) weighted distribution of proportion of sinistrals in *A. inversus* on Pulau Kapas.



3

Correlation between predation and density

Graph showing population density in *A. inversus* on Pulau Kapas in relation to predation ($r = -0.65$; $P < 0.001$).

