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

Menno Schilthuizen[1,2], **Sylvia Looijestijn**[3], **Sek Chuan Chua**[4], **and Ruth F. Castillo Cajas**[5]

[1]Naturalis Biodiversity Center, Leiden, the Netherlands
[2]Institute Biology Leiden, Leiden University, Leiden, the Netherlands
[3]Independent field biologist, Oranjelaan 35, 2635 JK, Den Hoorn, the Netherlands
[4]Independent field biologist, P.O. Box 300, Bukit Batok Central, 916510 Singapore
[5]Department of Animal Ecology and Tropical Biology, University of Würzburg, Germany

ABSTRACT

One of the very few snail taxa that display genetic antisymmetry (that is, roughly even mixes of genetically determined clockwise and anticlockwise 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 varies from 0.30 to 0.85. Density and overall predation are inversely correlated, but neither predicts PropS. Vegetation parameters also do not correlate with the proportion of sinistrals. We do, however, find a negative correlation between the predation rate on sinistrals and PropS, a finding which may warrant further study.

Keywords: left-right asymmetry, predation, sexual selection, vegetation structure, density, population genetics

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 [20] . 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 [27, 9]. The best example for this latter category comes from the Southeast-Asian genus *Amphidromus* (Camaenidae) [1]. This genus of large, colourful tree snails consists of several subgenera [25, 13], 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 [24, 19].

Since in most land snail species, copulation between mirror-imaged individuals is impeded [8, 1], chiral dimorphism is expected to be an evolutionarily unstable situation [26]. Therefore, over the past decade, the chiral dimorphism in *Amphidromus* has received much attention [19, 24, 7, 16], especially in the species *A. inversus* (Fig. 1). A series of studies has shown that, like in other pulmonate snails [20, 5, 15], the coiling direction is determined by delayed, single-gene inheritance and full dominance [19, 22]. Chiral dimorphism is present at all spatial scales [19], and S:D mixes are found even in individual trees [23]. No indication of niche differentiation can be observed [21], and dispersal is equal between both morphs and sufficient to assure the mixing of populations at a spatial scale larger than that of individual

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trees [23, 21]. These observations suggest that balancing selection must be maintaining the dimorphism. However, observations on predation revealed no indication of preference towards the commoner morph [19]–a situation which could have provided such balancing selection.

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 [22]. In fact, a slight preference appears to exist for dextrals to mate with sinistrals and vice versa. Moreover, the morphology of the spermatophore, the spermatophore-receiving organ, and the oviduct is 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 [19].

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, usually deviations from 50:50 proportions are 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 and co-workers [24] 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 same population over periods of up to ten years. Similarly, Craze [7] found a deviation in one *A. martensi* population in Borneo. In 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 percent sinistrals [19].

Craze [6] suggested that, under a fragmented population structure, frequent 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 at low densities and under environmental conditions that reduce interdemic 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 understorey ground cover.

We performed exploratory linear regressions among S:D proportions, density, predation rate, relative predation on sinistrals and vegetation characteristics. We then conducted inverse distance weighted interpolation in R using the total sample dataset. We first compared the performance of linear models calculated for each plotted variable against density, both when using the whole dataset (including low density numbers, n = 56) or only those samples in which at least 5 dead individuals were found (n = 40). We ran a simulation with 1,000 models for each scenario using the test set method (in which the remainder 30 percent of the data is used to calculate predictions made on a model with 70 percent of the data randomly selected) and calculated the Root-Mean-Square Error (RMSE) obtained from the predictions. The models from the smaller dataset performed worse than those using the whole dataset (for the predation rate: the mean values of RMSE for the whole dataset was 35.78587 and for the reduced dataset 42.58489, Welch's t-test(1958.75) = -15.51, p¡0.001. For the proportion of sinistral snails similar results were found: the mean values of RMSE for the whole dataset was 35.88 and for the reduced dataset 39.84, t-test(1982.37) = -9.5, p¡0.001).

However, to correct for the fact that our sample number was relatively small (n = 56), and that low densities might have a disproportionally 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 meters 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. To conduct all analysis and plots we used R version 3.0.2 and the following packages: sp [18, 4], maptools [2], rgdal [12], gstat [17], rgeos [3], plotKML [10] and plotrix [14].

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. 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 [19].

Mapping of density, predation, and PropS reveals considerable geographic variability. Density was high on parts of the west coast, and low elsewhere (Fig. 2). Predation showed the reverse pattern: low on parts of the west coast, high elsewhere (Fig. 3). Indeed, density and predation are significantly negatively correlated (r = -0.65; P ¡ 0.001; Fig. 4). Since the high-density/low-predation areas coincide with low-lying areas with a soil composed mainly of calcium-rich coral sand, we assume that the calcium content of the soil is what increases the snail densities 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.

Neither density nor predation, however, show a significant relationship with PropS (Student's T-test of correlation coefficients; P = 0.11 and P = 0.77, respectively; Fig. 5). PropS varies across the island, but mainly lies between 0.6 and 0.7, with a few pockets of higher and lower values. We could not find any significant relationship for PropS with field-recorded density, predation rate, canopy height, vine density, and ground cover. We did, however, find a significant negative correlation (r = -0.25; $P = 0.03$) with relative predation rate on sinistrals (proportion predation in empty sinistral shells / proportion predation in empty dextral shells). In other words, the lower the predation rate on sinistrals, the higher the relative frequency of sinistrals.

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 (Fig. 6). However, we did not find this geographic variation to be related to factors that may influence populations structure (i.e., density and vegetation characteristics), nor is there a clear geographic pattern to it. Therefore, our results do not support the hypothesis [6] that deviation from 50:50 proportions is due to genetic drift.

However, in our study we did not explore all potential deteminants of population structure. The vegetation characteristics that we measured were estimated by the researchers in the field, and a GIS study with LiDAR data [11] might reveal patterns that have remained hidden in the present study. On the other hand, our finding of a (weak) negative correlation between PropS and relative predation on sinistrals may warrant further study as well. We do not yet have a plausible hypothesis for why reduced predation may affect only one enantiomorph, nor how this would then increase PropS beyond 0.5, but it is clear that our understanding of the ecological genetics of chirality in this species is not yet complete.

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FIGURES

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

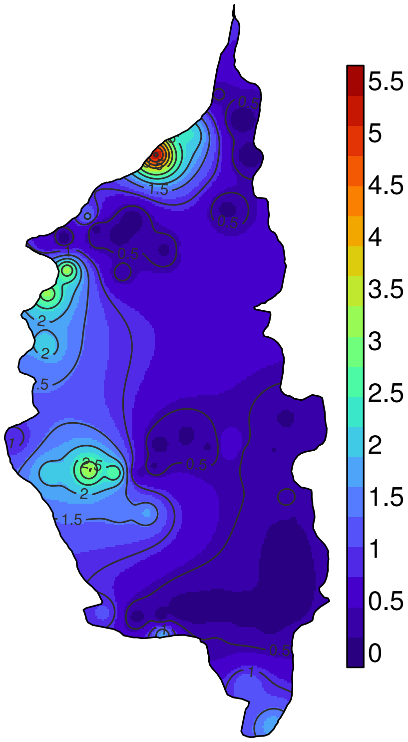

Figure 2. Map showing the density distribution of *A. inversus* on Pulau Kapas (unit = snails per person-minute search time)

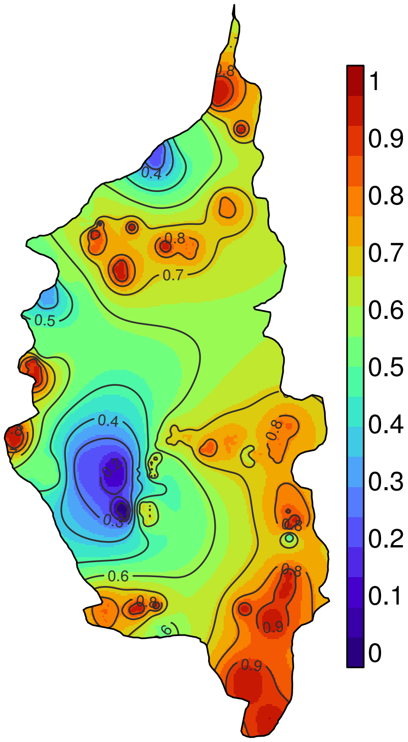

Figure 3. Map showing the weighted predation rates in *A. inversus* on Pulau Kapas (proportion of empty shells with predation damage)

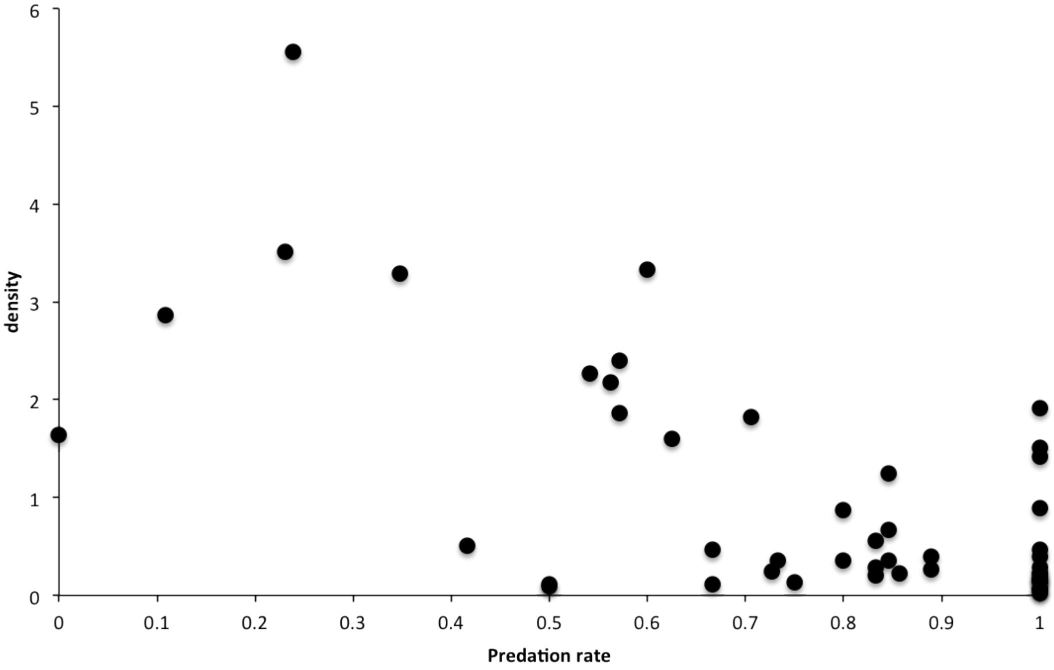

Figure 4. Graph showing population density in *A. inversus* on Pulau Kapas in relation to predation (r = -0.65; P¡0.001)

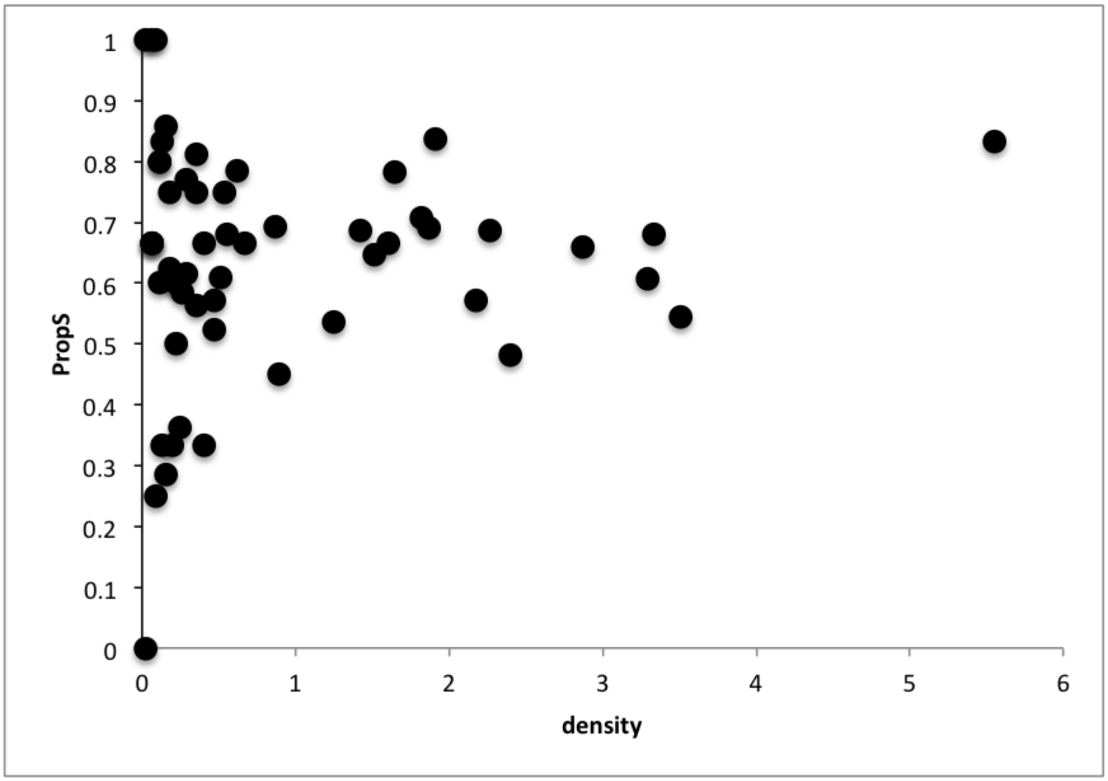

Figure 5. Graph showing proportions of sinistrals in relation to density (N.S.)

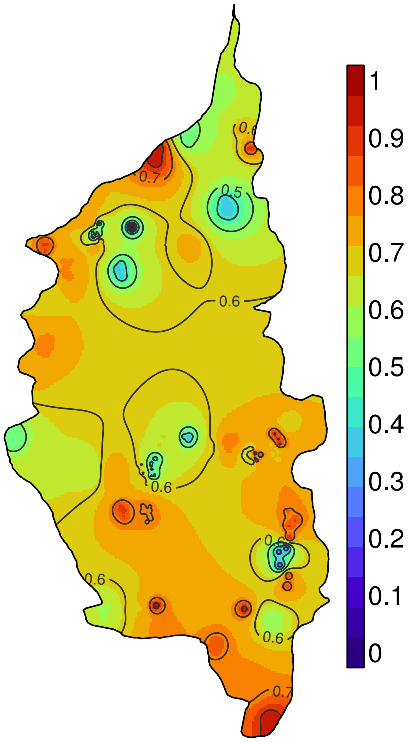

Figure 6. Map showing the weighted distribution of proportion of sinistrals in *A. inversus* on Pulau Kapas